Pharmacokinetics and pharmacodynamics of the psychedelic compound DMT

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Cover illustration: Varying degrees of hemolysis in human plasma samples. Photo by Emma Eckernäs.

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“Kliv, kliv, överlev”

Mathias Fredriksson

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ABSTRACT

N,N-dimethyltryptamine (DMT) is a psychedelic compound that is being investigated as a treatment option in depression. It is also being used as a research tool in research aiming to investigate the neurobiology of the human consciousness using brain imaging techniques. However, despite the increasing research on DMT, much remains to be known about its pharmacokinetic and pharmacodynamic properties. Increasing this understanding is essential in assuring safe and efficacious use of DMT in the future.

The aim of this thesis was to investigate the pharmacokinetics and pharmacodynamics of DMT as well as to use the newly gained knowledge to design new dose regimens. A liquid chromatography tandem mass spectrometry method was developed and validated to enable quantification of DMT and two of its metabolites in biological samples. Nonlinear mixed effects modeling was used to describe the pharmacokinetics and pharmacodynamics of DMT using data obtained from two clinical studies. The models were used to provide dose recommendations for administering DMT as a continuous intravenous infusion. A more individualized dose regimen, based on observed psychedelic intensity ratings, was also developed. The metabolism of DMT *in vitro* was assessed using human liver microsomes as well as recombinant cytochrome P450 enzymes. These experiments showed that DMT is a substrate for CYP2D6 and that this likely leads to formation of hydroxylated metabolites.

Overall, this thesis provides new knowledge on the pharmacokinetics and pharmacodynamics of DMT. It also presents novel dosing strategies and demonstrates how pharmacokinetic and pharmacodynamic modeling and simulation can be used to further optimize DMT dosing in future clinical studies.

Keywords: N,N-dimethyltryptamine, psychedelic, nonlinear mixed effects modeling, pharmacokinetics, pharmacodynamics

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SAMMANFATTNING PÅ SVENSKA

Trots att ca 300 miljoner människor världen över lider av depression eller ångest är tillgången på effektiva läkemedelsbehandlingar fortfarande bristfällig. En stor andel av de som behandlas med dagens standardläkemedel får ingen eller liten effekt av behandlingen. Bristen på effektiva behandlingar har lett till ett ökat intresse för så kallade psykedeliska substanser som möjliga behandlingsalternativ. Exempel på psykedeliska substanser är LSD och psilocybin, varav den senare utgör den aktiva substansen i vissa psykedeliska svampar. Dessa substanser har effekter på hjärnan som bland annat kan leda till hallucinationer och andra sinnesförändringar. Forskning har visat att patienter som lider av depression upplever en förbättring, som ibland håller i sig över flera månader, efter bara en eller ett par behandlingstillfällen med psykedeliska substanser.

Ytterligare en psykedelisk substans som väckt stort intresse inom forskningsvärlden är N,N-dimetyltryptamin, även kallad DMT. DMT har visat sig ha positiva effekter i patienter med depression. Substansen används även i forskning som undersöker vad som händer i hjärnan när man har en psykedelisk upplevelse. Tanken är att det ska hjälpa forskare att förstå vilka processer i hjärnan som ligger bakom det vi kallar för det mänskliga medvetandet. Trots att antalet studier med DMT ökar, saknas fortfarande en grundläggande förståelse för hur substansen beter sig i människokroppen. För att kunna använda ett läkemedel på ett så effektivt och säkert sätt som möjligt behövs en förståelse för hur läkemedlet tas upp och bryts ner i kroppen, läkemedlets så kallade farmakokinetik, samt hur de här processerna påverkar läkemedlets effekt, den så kallade farmakodynamiken. Väldigt lite forskning har hittills gjorts för att undersöka DMT:s farmakokinetik och farmakodynamik.

I den här avhandling presenteras nya fynd kring farmakokinetiken och farmakodynamiken för DMT. Majoriteten av forskningen som presenteras här är resultatet av ett samarbete med en forskargrupp vid Imperial College London. I London har man gjort kliniska studier där DMT har injicerats intravenöst till friska frivilliga. Man har samlat blodprover från studiedeltagarna, för att kunna mäta koncentrationen DMT i blodet över tid, samt mätt effekterna av DMT. Detta har dels gjorts med hjälp av EEG samt genom att be deltagarna rapportera, på en skala 0-10, hur intensiv den psykedeliska upplevelsen var. Avhandlingen presenterar en metod för att kunna mäta DMT-koncentrationer i de insamlade blodproverna samt matematiska modeller för att beskriva hur koncentrationerna i blodet förändras över tid och hur detta styr de observerade effekterna av DMT. Modellerna kan sedan användas för att förutsäga exempelvis vad effekterna skulle bli om man ändrar dosen DMT. I avhandlingen presenteras exempel på hur modellerna har

använts i praktiken för att avgöra vilken dos som ska användas i en av de kliniska studierna. Ytterligare ett exempel presenteras på hur man kan använda en av modellerna för att designa individanpassade doseringsscheman för DMT i framtiden. Detta kommer troligtvis bli nödvändigt då både koncentrationerna och effekterna varierar stort mellan olika individer som har fått samma dos DMT.

Utöver detta presenteras även en studie över hur DMT bryts ner i olika cellinjer. I kroppen finns en mängd olika läkemedelsmetaboliserande enzymer (proteiner som har förmåga att bryta ner främmande substanser). Det är viktigt att känna till vilka enzymer som är ansvariga för att bryta ner ett läkemedel eftersom det kan påverka exempelvis vilka andra substanser ett läkemedel kan kombineras med på ett säkert sätt. Resultaten av detta visar att DMT bryts ner av ett enzym vid namn CYP2D6 och att detta leder till bildandet av nedbrytningsprodukter som potentiellt skulle kunna ha en egen farmakologisk aktivitet, och därmed påverka effekten av att administrera DMT. Mer forskning behövs för att studera om dessa nedbrytningsprodukter även kan bildas i människokroppen efter administrering av DMT och hur det i så fall påverkar effekten och risken för biverkningar av DMT.

Sammanfattningsvis presenterar den här avhandlingen nya fynd kring farmakokinetiken och farmakodynamiken för DMT samt hur dessa kan användas för att optimera doseringen av DMT i framtida studier. Förhoppningen är att detta kommer leda till en mer effektiv utveckling av DMT som ett potentiellt framtida behandlingsalternativ mot exempelvis depression.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. **Eckernäs E**, Bendrioua A, Cancellerini C, Timmermann C, Carhart-Harris R, Hoffmann K-J, Ashton M. Development and application of a highly sensitive LC-MS/MS method for simultaneous quantification of N,N-dimethyltryptamine and two of its metabolites in human plasma. *Journal of Pharmaceutical and Biomedical Analysis,* 2022;212:114642.

II. **Eckernäs E**, Timmermann C, Carhart-Harris R, Röshammar D, Ashton M. Population pharmacokinetic/pharmacodynamic modeling of the subjective psychedelic experience induced by N,N-dimethyltryptamine – implications for dose considerations. *Clinical and Translational Science,* 2022;15(12):2928-2937

III. **Eckernäs E**, Timmermann C, Carhart-Harris R, Röshammar D, Ashton M. N,N-dimethyltryptamine affects electroencephalography response in a concentration dependent manner – a pharmacokinetic/pharmacodynamic analysis. *CPT:Pharmacometrics and Systems Pharmacology,* 2023;12(4):474-486.

IV. **Eckernäs E,** Macan-Schönleben A, Andresen-Bergström M, Birgersson S, Hoffmann K-J, Ashton M. N,N-dimethyltryptamine forms oxygenated metabolites via CYP2D6 - an in vitro investigation. *Submitted*

V. **Eckernäs E**, Koomen J, Timmermann C, Carhart-Harris R, Röshammar D, Ashton M. Optimized infusion rates for N,N-dimethyltryptamine to achieve a target psychedelic intensity based on a modeling and simulation framework. *CPT:Pharmacometrics and Systems Pharmacology,* 2023;12(10):1398-1410

VI. **Eckernäs E**, Luan L, Timmermann C, Carhart-Harris R, Röshammar D, Ashton M. Using pharmacokinetic/pharmacodynamic modeling and simulation to design individually tailored infusion rates for an extended DMT experience. *In manuscript*

CONTENT

ABBREVIATIONS

5-HT	5-hydroxytryptamine
ADME	Absorption, distribution, metabolism and excretion
CYP	Cytochrome P450
DMT	N,N-dimethyltryptamine
DMT-NO	N,N-dimethyltryptamine N-oxide
$EC_{50,e}$	Concentration required in the effect compartment to reach 50% of the maximum achievable stimulatory effect
EEG	Electroencephalography
E_{max}	Maximum achievable stimulatory effect
FDA	Food and Drug Administration
HLM	Human liver microsome
HR-MS	High resolution mass spectrometry
IAA	Indole 3-acetic acid
$IC_{50,e}$	Concentration required in the effect compartment to reach 50% of the maximum achievable inhibitory effect
I_{max}	Maximum achievable inhibitory effect
k_{e0}	Effect compartment equilibration rate
LC-MS/MS	Liquid chromatography tandem mass spectrometry

LSD	Lysergic acid diethylamide
MAO	Monoamine oxidase
MDMA	Methylenedioxymethamphetamine
NADPH	nicotinamide adenine dinucleotide phosphate
NMR	Nuclear magnetic resonance
OFV	Objective function value
TAAR1	Trace amine-associated receptor 1
UPLC	Ultra-performance liquid chromatography
γ	Hill factor describing the sigmoidicity of an exposure-response relationship

DEFINITIONS IN SHORT

Clearance	the capacity to eliminate a drug, defined as the total volume of blood plasma that is cleared from drug per unit of time
In vitro	studies performed outside of the normal biological context, i.e., in labware
In vivo	studies conducted in living organisms including animals and humans
Pharmacodynamics	the study of how drugs effect the body
Pharmacokinetics	the study of the time course of drug concentrations in the body, including how the drug is absorbed, distributed in and eliminated from the body
Pharmacometrics	a field of science that uses mathematical models to describe biological and pharmacological data
Psychedelics	drugs which primary effect is to induce altered states of consciousness through agonism at serotonin receptors
Volume of distribution	a theoretical volume describing how much a drug distributes from blood plasma into other tissue

1 INTRODUCTION

Depression and anxiety disorders are two of the largest contributors to non-fatal health losses worldwide, with approximately 300 million people estimated to suffer from one or both of these conditions [1]. However, despite the huge impact on human lives, there is currently a lack of efficacious treatments. A large proportion of patients do not respond to standard treatment options and are considered treatment resistant [2, 3]. Recently, interest in serotonergic psychedelics has increased as they have shown potential as treatment options in a number of psychiatric disorders, including depression and some anxiety disorders [4-15]. Several smaller clinical studies have demonstrated both acute and long-term effects after only a single or few administrations.

One psychedelic compound of particular interest is N,N-dimethyltryptamine (DMT). This compound has been studied in the clinic both on its own, mainly through intravenous administration [10, 16-21], or as part of the traditional Amazonian brew ayahuasca [7, 9, 22-26]. However, despite the positive indications of DMT as a future treatment option, much of the knowledge that is usually gathered early in the clinical development of a new drug compound is still lacking for DMT. This includes a basic understanding of its pharmacokinetics and pharmacodynamics. An understanding that is essential in assuring safe and efficacious use of DMT in future clinical studies.

This thesis is based on six subprojects and presents new knowledge on the pharmacokinetics and pharmacodynamics of DMT based on data from clinical studies with DMT performed in healthy subjects at Imperial College London [16, 19]. The metabolic pathways of DMT are also investigated using *in vitro* assays. Finally, using a modeling and simulation framework, it is demonstrated how this knowledge can be used to design new dose regimens and guide future clinical studies with DMT.

1.1 PSYCHEDELICS

1.1.1 SEROTONERGIC PSYCHEDELICS

Psychedelics are a class of psychoactive drugs whose main effect is to induce altered states of consciousness, including changes in perception, mood and cognitive processes [27]. The term psychedelic is sometimes used interchangeably with “hallucinogen”. However, while hallucinogens may also include compounds such as cannabis, ketamine and methylenedioxy methamphetamine (MDMA), the term psychedelic refers exclusively to

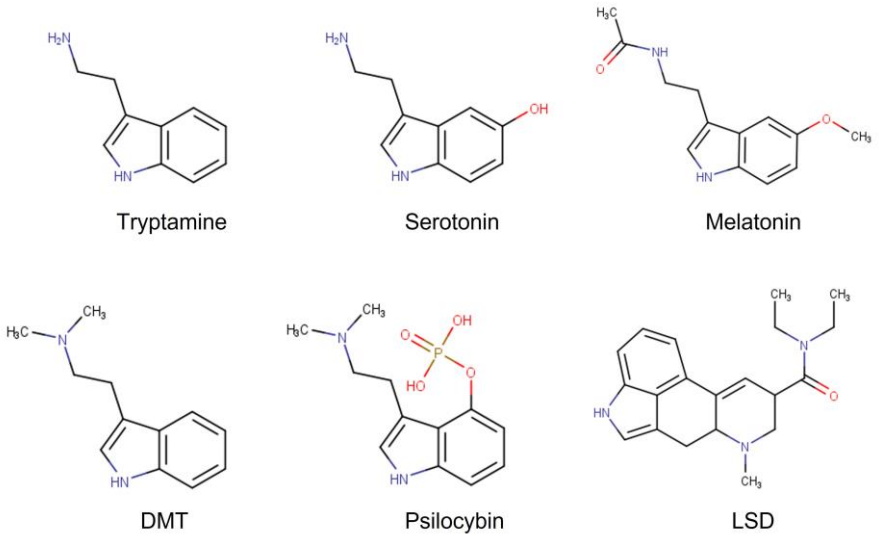


Figure 1. Molecular structures of different psychoactive and non-psychoactive tryptamine derivatives.

compounds which exert their effect primarily through agonism at serotonin receptors; so called classic serotonergic psychedelics.

This group of compounds can be divided into two different subgroups – tryptamine and phenethylamine psychedelics. Phenethylamine psychedelics include compounds such as mescaline which can be found in various types of cacti. The tryptamine group includes, for example, lysergic acid diethylamide (LSD), psilocybin and DMT (Figure 1). Many of these compounds are naturally occurring and have traditionally been used as part of religious practices in ancient cultures.

Serotonergic psychedelics act mainly as agonists at serotonin receptors in the brain [27]. Their affinity for different serotonin receptors may vary, but the psychedelic effects are generally attributed to agonism at the 5-hydroxytryptamine (5-HT) 2A receptor [28, 29]. However, exactly how activation of these receptors leads to a psychedelic experience is not entirely known. Furthermore, preclinical investigations indicate that the 5-HT2A receptor may also mediate the therapeutic effects of psychedelics [30].

1.1.2 THERAPEUTIC POTENTIAL OF PSYCHEDELICS

Recently, the interest in research with psychedelics has increased as they have shown potential as therapeutic options in a number of psychiatric disorders. Psilocybin is perhaps the most well-studied, and several studies show positive

effects in patients suffering from major depressive disorder [4, 5, 11, 13, 15]. Other indications that have been studied include substance abuse [31] and anxiety in end-stage cancer [6, 8, 12, 14]. Most of these studies include only one or two administrations of a psychedelic compound. Still, positive effects have been observed up to six months after administration [4].

Administration of psychedelics to patients is usually combined with some form of psychotherapy, commonly referred to as psychedelic-assisted psychotherapy. It has been hypothesized that psychedelics induce a so called relaxation of prior beliefs and that this makes the subject more perceptive to psychotherapy [32]. However, whether the psychedelic experience is necessary or not to achieve therapeutic benefits is a source of disagreement within the field [33]. Many study participants refer to their psychedelic experience as being among the most meaningful in their lives [6, 8]. Hence, some people argue that the prominence of the subjective psychedelic effects is what helps the patient to, for example, change certain thought patterns and that it is therefore an essential aspect of the therapy [34]. In contrast, others believe that the therapeutic benefit stems from changes in the brain that could be separated entirely from the psychedelic effects [35]. It has been proposed that these hypotheses could be tested through administration of psychedelics to subjects under general anesthesia or by co-administration with a 5-HT2A antagonist [33, 35, 36].

Furthermore, while most studies show therapeutic benefits, the interpretation of the results is not straightforward [37]. Due to the subjective experience produced by these compounds, blinding is difficult within this context. Even though most studies include a placebo arm, both study participants and clinicians are in many cases able to correctly guess whether they have received placebo or the active drug [6, 7]. This makes it difficult to distinguish potential expectancy effects from actual pharmacological effects [38]. Consequently, clinical trials with psychedelic compounds needs to be well-designed to avoid bias in the interpretation of outcomes [39, 40].

1.1.3 NEUROIMAGING STUDIES WITH PSYCHEDELICS

In addition to the research on potential therapeutic benefits, psychedelics have been used in research aiming to understand the neurobiology of the human consciousness [16, 26, 41-47]. The unique features of these compounds make them suitable research tools in this context. Using different brain imaging techniques, the idea is to investigate what happens in the brain during a psychedelic experience. These data can then be used to draw conclusions about how changes in brain signaling may be coupled to changes in, for example, perception and cognition.

One commonly used technique in this field of research is electroencephalography (EEG). EEG measures electrical signaling in the brain through electrodes placed on the scalp of the study participant. This generates data with high temporal resolution, however, since the measurements occur on the scalp, spatial resolution is not as high. The data produced with EEG can be processed and summarized in many different ways. One common way to evaluate the effects of psychedelics is to look at changes in different frequency bands. When neurons fire synchronously this can give rise to oscillatory activity, commonly referred to as brainwaves. These brainwaves can be divided into different frequency bands based on the wavelength: delta (0.5-4 Hz), theta (4-8 Hz), alpha (8-12 Hz), beta (12-30 Hz) and gamma (>30 Hz). The different frequency bands can to some degree be coupled to brain function. For example, alpha power has been linked to information processing and semantic orientation [48].

While the effects vary slightly between different studies and compounds, psychedelics have been shown to robustly decrease alpha power [16, 26, 41-46]. Furthermore, psychedelics have been shown to increase signal diversity which can be seen as a measure of brain entropy [16, 41]. Increased signal diversity could be loosely translated to mean more random signaling in the brain. High signal diversity is generally coupled to a "higher level of consciousness", i.e., a more awake state [16, 49, 50]. Exactly how these changes in brain signaling are coupled to the subjective experience is, as of yet, inconclusive. However, blocking of the 5-HT2A receptor has been shown to inhibit the reductions in alpha power induced by ayahuasca, indicating that this effect is also mediated via 5-HT2A receptors [45].

1.2 DMT

1.2.1 HISTORY OF DMT

DMT is a serotonergic psychedelic compound that has traditionally been used by indigenous people in South America as part of the Amazonian brew ayahuasca, a Quechua word meaning "vine of the soul" [51]. This brew is made by boiling the leaves of the *Psychotria viridis* plant, which contains large amounts of DMT, and the bark of the *Banisteriopsis caapi* vine, which contain harmala alkaloids that enable oral absorption of DMT. In 2006, the use of ayahuasca for religious purposes was protected under the Religious Freedom Restoration Act in the US [52].

While there is evidence suggesting that ayahuasca has been used for centuries [53], the role of DMT as the active ingredient was discovered during the 1900's. In 1931, DMT was synthesized for the first time by Canadian chemist

Richard Manske [54]. However, it was only in 1956 that Hungarian chemist Stephen Szàra discovered the psychedelic potential of these compounds through experiments on himself [55]. This was shortly after the discovery of the effects of LSD in 1943 and these discoveries subsequently led to an era of intense research with psychedelics during the 1950's and 1960's. While promising results were obtained for psychedelics as therapeutic options, the spread of psychedelics as recreational drugs in the general population led to increased concern from different authorities. In 1970, psychedelics, including DMT, were classified as Schedule 1 drugs under the Controlled Substances Act, meaning that they were considered highly addictive with little or no medical value. This made it very difficult to continue clinical research with psychedelics and the field suffered a major hit back with practically no clinical studies being performed during the 1970's and 1980's.

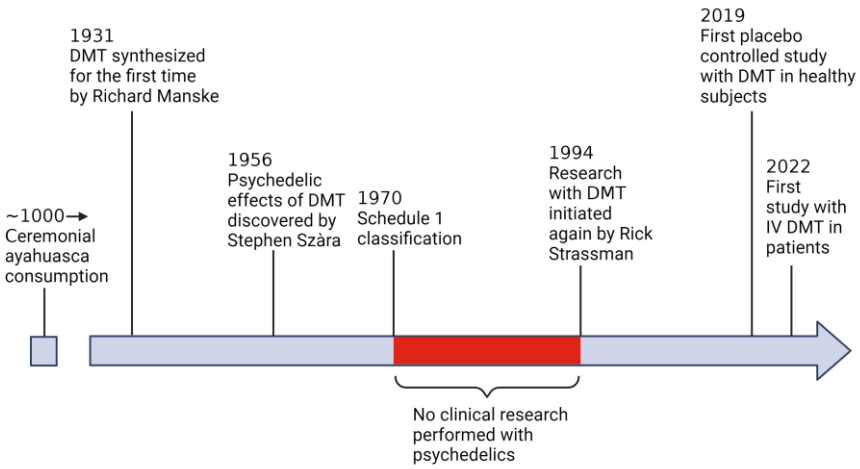


Figure 2. Timeline over significant events in the history of DMT. Created with BioRender.com.

In 1994 however, Rick Strassman published the first clinical study performed with a psychedelic compound for decades [17, 18]. This was a dose escalation study with DMT, investigating the effects and tolerability in healthy subjects. This became the starting point for a new era of psychedelic research. Since then, the field has had a huge surge, with multiple centers for psychedelic research being established and several companies being founded, focusing on the development of psychedelics as drug candidates. The choice of DMT as

the focus of Strassman's studies in the 1990's may have been a key factor in this revival. Strassman himself has stated that he chose DMT in particular because it was less well known and would not draw as much attention and potential resistance as for example LSD [56].

1.2.2 PHYSICOCHEMICAL PROPERTIES OF DMT

DMT is a small, lipophilic molecule with a molecular weight of 188 g/mol and a logP of 2.57 [57]. It is a weak base and in its free base form, DMT is poorly soluble in water. Hence, for the purpose of parenteral administration to humans, DMT is commonly prepared as a fumarate salt. Since fumaric acid has two carboxyl functional groups, this salt can be prepared either at a 1:1 or 2:1 ratio, the latter being referred to as a hemifumarate salt.

Structurally, DMT is an analogue of tryptamine, a metabolite of the essential amino acid tryptophan. They differ only in that DMT is demethylated on one of the nitrogens (Figure 1). The tryptamine core is also shared with endogenous neuromodulators such as serotonin and melatonin.

1.2.3 TARGETS AND PHENOMENOLOGY OF DMT

While it has been shown that psychedelics exert their psychoactive effects mainly through agonism at 5-HT2A receptors [28, 29], the exact number and nature of their targets may vary. In addition to 5-HT2A, DMT has affinity for 5-HT1A, 5-HT1B, 5-HT1D, 5-HT2B, 5-HT2C, 5-HT6, and 5-HT7 receptors [58]. However, the effects of binding to 5-HT2A are the most well characterized and has been shown to promote cortical neuron growth and spinogenesis [59]. It has been hypothesized that this neuroplastic effect is part of what underlies the antidepressant effects of psychedelics [60]. Interestingly, recent studies indicate that this effect is mediated through activation of intracellular 5-HT2A receptors [61]. This would explain why similar effects are not produced by serotonin itself, as it is incapable of crossing the cell membrane to the same degree as for example DMT. It has also been shown that the 5-HT2A receptor does not desensitize to DMT over time [62].

In addition to 5-HT receptors, DMT is known to be an agonist of the sigma-1 receptor [63]. However, the affinity is around 100-fold lower than for 5-HT2A receptors. The sigma-1 receptor has been associated with anti-depressive features [64, 65], and it is possible that the binding of DMT to this receptor plays a role in its observed effects. DMT has also been shown to be a substrate for trace amine-associated receptor 1 (TAAR1) [66].

The varying target affinities of serotonergic psychedelics may explain the different phenomenology of the psychedelic experience observed after administration of different psychedelic compounds. The psychedelics effects

of DMT are generally described as being more unpredictable than those of, for example, psilocybin [56]. The DMT experience may include visual, auditory as well as sensory hallucinations and it is not uncommon that subjects report feelings of dissolution of time and space or being transported to an alternate world [56, 67, 68].

1.2.4 THERAPEUTIC POTENTIAL OF DMT

Although being relatively few, there are both preclinical and clinical studies indicating a role for DMT as a therapeutic candidate. Most clinical studies with DMT have been performed through the administration of ayahuasca [7, 9, 69]. These studies have shown decreased depression rates as compared to placebo. However, the complexity of the ayahuasca brew makes it difficult to draw conclusions about the specific role of DMT in the anti-depressive effects. Recently, the first study investigating DMT alone in patients suffering from depression was published [10]. While this was a pilot study with a small study sample, results did show decreased depression rates in patients treated with DMT. While these results are still preliminary, there are also preclinical research indicating an anti-depressive action of DMT. DMT has, for example, been shown to increase swim time in a forced swim test in rats, a common preclinical model of depression [70].

1.2.5 ENDOGENOUS ROLE FOR DMT

In contrast to many other psychedelic compounds, DMT is believed to be produced endogenously in humans and has been measured in small amounts in various body fluids and tissues [71-73]. However, the physiological role of endogenous DMT is not known. Numerous hypotheses have occurred throughout the years. When DMT was first discovered it was described as a 'schizotoxin' and it was hypothesized that it might play a role in the development of schizophrenia [74]. This idea has since then been discarded as several studies failed to detect elevated levels of DMT in patients with schizophrenia. Furthermore, as the psychedelic experience has similarities with dream states as well as mystical and near-death experiences, it has been hypothesized that such experiences may be explained by endogenous release of DMT [56, 75]. However, there is little evidence to support this hypothesis.

Another aspect is that the levels of DMT detected in blood are very low. For DMT to have any physiological relevance, much higher concentrations would likely be needed. It has therefore been suggested that DMT is produced and stored at higher concentrations locally in the body [76]. Some even go so far as to suggest that the proposed pathway for DMT transport into and storage in the brain would indicate an importance to the brain similar to that of glucose [77]. However, there is no *in vivo* data to support this claim. Hence, it remains

to be clarified where in the body DMT is produced, if the amounts of DMT in the body are high enough to have any biological impact and, if so, what that impact may be [78].

1.3 PHARMACOKINETICS AND PHARMACODYNAMICS

Pharmacokinetics is the study of what happens to the drug in the body whereas pharmacodynamics is the study of what the drug does to the body. The pharmacokinetics of a drug are usually characterized by measuring drug concentrations in blood plasma over time.

Four separate processes determine the pharmacokinetics; the absorption, distribution, metabolism and excretion of the drug (ADME). For a drug to be able to exert its effects, it must first reach its site of action, often via the systemic circulation. The fraction of the drug that reaches the systemic circulation is described by the bioavailability, which is partly determined by how much of the drug is absorbed from the site of administration into the system. The extent of absorption differs depending on the drug as well as the route of administration. For intravenously administered drugs, the bioavailability is 100% as it is administered directly into the systemic circulation. Drugs that are orally administered on the other hand need to be absorbed over the gastrointestinal wall and pass the liver without being metabolized before reaching the systemic circulation. This normally leads to a lower bioavailability as compared to intravenously administered drugs. Once the drug has reached the circulation, it can distribute from there to other parts of the body. The extent of this distribution is generally determined by the lipophilicity of the drug as well as the ability to bind to different moieties in the body. Elimination of drugs from the body occurs via two major pathways. One pathway is through metabolism, i.e., enzymatic degradation. This mainly occurs in the liver where drug metabolizing enzymes are expressed. The other pathway is through excretion via the kidneys. This is most common for more water-soluble compounds. The purpose of drug metabolism is to make the compounds more water soluble so that they can subsequently be excreted via the urine by the kidneys. Together, the ADME processes control how the concentrations of a drug in the body change over time. These changes can be described mathematically by a number of pharmacokinetic parameters such as clearance, which describes the elimination capacity, and volume of distribution, which describes how much the drug distributes from plasma into other tissue.

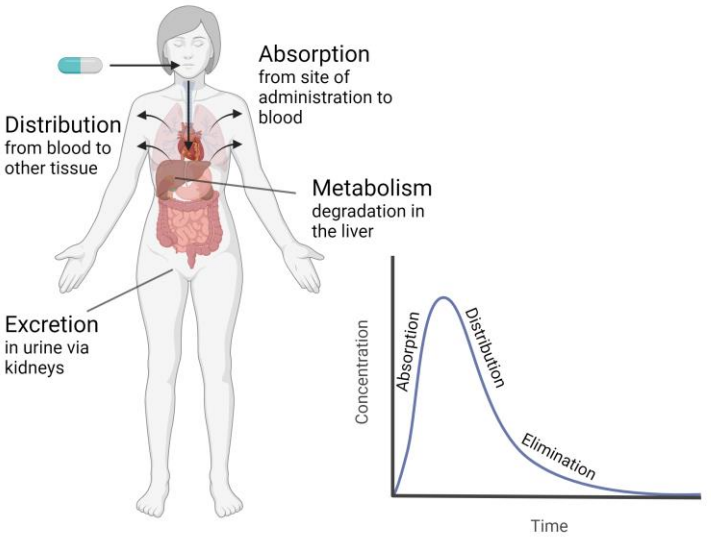


Figure 3. Overview of the ADME processes; absorption, distribution, metabolism and excretion. Created with BioRender.com.

The pharmacodynamics of a drug includes both desirable and undesirable effects. Usually, a relationship exists between the concentrations of the drug in the body and the observed effect, referred to as an exposure-response relationship. By characterizing this relationship, one can establish what concentrations are needed to reach a desired effect while also avoiding potential adverse effects. If such a therapeutic target has been established, this can be used to decide what dose to administer and how often it should be administered.

1.3.1 PHARMACOKINETICS/PHARMACODYNAMICS OF DMT

When administered on its own, DMT is not orally available due to extensive metabolism by the enzyme monoamine oxidase A (MAO-A) before DMT can reach the systemic circulation [79]. This is one of the main reasons why many researchers have chosen to investigate ayahuasca rather than DMT alone. Ayahuasca, in addition to DMT, contain harmala alkaloids, such as harmine, which act as MAO-A inhibitors, thereby making DMT available to the systemic circulation [22, 80]. Studies aiming to investigate DMT alone have instead used parenteral injections [16, 17, 19, 20, 81]. However, as MAO-A is an enzyme that is present throughout the entire body, DMT is metabolized rapidly also after parenteral administration. Previous studies have shown that DMT has a half-life of approximately 10 minutes after intravenous

administration [17]. As the psychedelic effects of DMT appear to follow the plasma concentrations rather closely [16, 18], this means that both the exposure to and the effects of DMT after an intravenous bolus administration are short-lived. Administration of ayahuasca leads to prolonged exposure with effects lasting for several hours [82].

Metabolism by MAO-A results in the formation of indole 3-acetic acid (IAA), an inactive metabolite that is excreted in urine. This is the main metabolite of DMT and the only one that has been detected in any larger amounts after intravenous administration [83]. However, combining DMT with MAO inhibitors appears to cause a shift in the metabolic pathways, and several other metabolites have been identified after administration of ayahuasca. While IAA is still the most prominent metabolite, an N-oxidated metabolite (DMT-NO) has also been detected in substantial amounts [23]. Other potential metabolites that have been identified in humans include a demethylated product and hydroxylated products [23, 84]. However, the complexity of the ayahuasca brew makes it difficult to conclude whether they are actual metabolites of DMT or not. Furthermore, it is not known which enzymes are involved in the formation of these metabolites. While DMT was recently shown to be a substrate for CYP2D6, no data on which metabolite is formed via this pathway was presented [20].

Interestingly, repeated dosing of intravenously administered DMT does not lead to any psychological tolerance [85]. This is in contrast to other psychedelics, such as LSD or psilocybin [86, 87]. Furthermore, DMT administered in a clinically controlled setting, is generally considered safe also at doses that cause intense psychedelic experiences. Adverse effects include acute cardiovascular effects such as a rise in heart rate and increased blood pressure [17]. However, these effects wear off quickly, and no long-term cardiovascular effects have been reported. In addition, DMT and other tryptamine psychedelics are not considered to be addictive [88].

The pharmacokinetic and pharmacodynamic properties of DMT has led to an interest for administering DMT as an infusion over a longer period of time, rather than as a bolus dose [89], and in the last couple of years, this has become reality with a couple of studies investigating this option [19, 21]. There are a number of reasons for wanting to explore continuous infusion of DMT. One aspect is to enable the study of the acute effects of DMT over a longer period of time, for example using EEG. Another aspect is to potentially increase the therapeutic effects of DMT by allowing a combination with psychotherapy. As previously mentioned, psychedelic administration is commonly combined with psychotherapy and many believe this to be an integral part of achieving the desired therapeutic effects. The short half-life of DMT makes this difficult after bolus administration as the effects may wear off as early as after 10

minutes in some cases. Other approaches to overcome the issues with DMT's short half-life has also been proposed, such as administration of chemically modified versions of DMT with altered pharmacokinetic properties [90].

1.4 BIOANALYSIS

Bioanalysis is a subfield of analytical chemistry that refers to the quantification of drugs or endogenous substances in biological samples. Having accurate and precise bioanalytical methods is essential to be able to characterize the pharmacokinetics of a drug.

Liquid chromatography tandem mass spectrometry (LC-MS/MS) is generally considered the gold standard method in bioanalysis. This method combines the separation of different compounds on a chromatographic column with specific detection based on fragmentation pattern and mass-to-charge ratio in the mass spectrometer. This enables highly sensitive detection and quantification of the analyte.

A schematic overview of the LC-MS/MS process can be seen in Figure 4. In brief, samples are injected onto a chromatographic column where the analytes are retained for differing lengths of time depending on the chemical properties of the analyte, the column and the mobile phase that flows through the column. Once the analyte elutes from the column, it moves into the tandem mass spectrometer, where it is ionized. In the first mass spectrometer, the ionized compound is separated and detected based on a mass-to-charge ratio. The so-called precursor ions are then forced to fragment into smaller product ions which, in turn, can be separated and detected based on their mass-to-charge ratio. The fragmentation step is the key to the high specificity of MS/MS as it allows the separation of compounds with similar molecular weights.

Before a sample can be analyzed using LC-MS/MS, some type of sample preparation is usually required. The purpose of sample preparation is to clean the sample to make it compatible with the analytical system and remove components that might interfere with detection. Common methods of sample preparation are for example protein precipitation, liquid-liquid extraction and solid-phase extraction

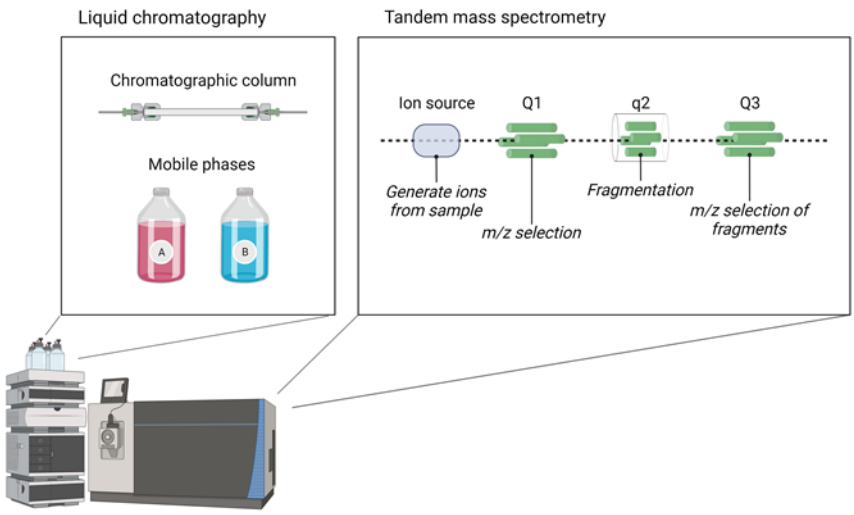


Figure 4. Schematic overview of an LC-MS/MS system. The analyte is first injected onto a chromatographic column where it is retained for differing lengths of time depending on the properties of the column and the mobile phase flowing through the column. The analyte then moves into the tandem mass spectrometer where it is ionized. The compound can be separated and detected based on the mass-to-charge ratio of the mother compound (Q1) and on the mass-to-charge ratio of specific fragments formed from the mother compound (Q3). Created with BioRender.com.

A number of methods for analyzing DMT in biological samples have been published. Older studies of DMT have generally employed gas chromatography with varying methods of detection [91, 92]. Since 2012 and onwards, several LC-MS/MS methods for quantification of DMT, and sometimes metabolites, have been published [83, 84, 93-96]. However, the sensitivity as well as the capability to also quantify metabolites vary between these methods. Furthermore, not all have been validated according to current regulatory standards.

1.5 *IN VITRO* METABOLISM

When a drug is eliminated mainly via metabolism, the metabolic pathways can affect the efficacy and safety of the drug. If the metabolic capacity is somehow altered, this may lead to changes in plasma concentrations of the drug and its metabolites, which could in turn affect the observed effect as well as the number of adverse events [97-99]. This can be caused either by changes in

concentrations of the drug itself or, in some cases, by active metabolites [100, 101]. There are several reasons why alterations in the metabolic capacity might occur. This could be either due to inhibition or induction of drug metabolizing enzymes by other drugs or due to genetic differences leading to variability in metabolic capacity between different individuals. Consequently, having a good understanding of the metabolic pathways of a drug is important in order to assure that the drug is safely administered, for example in terms of avoiding potential drug-drug interactions.

Cytochrome P450 (CYP) enzymes are the most common drug metabolizing enzymes, accounting for around 80% of all drug metabolism of registered medicines [102]. Drug-drug interactions on a metabolism level are usually caused by interactions involving CYP enzymes. Furthermore, some CYP enzymes have known polymorphisms leading to variability in metabolic capacity between different individuals [103, 104]. Consequently, an investigation of potential CYP metabolism of a new drug compound is required by the Food and Drug Administration (FDA) [105]. Initial investigations should preferably be performed *in vitro* as a way to elucidate potential metabolic pathways and risks for drug-drug interactions before moving into clinical studies.

1.5.1 REACTION PHENOTYPING

One aspect of understanding the metabolic pathways of a drug is the characterization of which CYP enzymes are capable of metabolizing the drug. This is commonly referred to as reaction phenotyping [106]. While reaction phenotyping can include a number of different analyses, two of the most common are incubations with recombinant enzymes as well as selective inhibition using human liver microsomes (HLM). Studying recombinant enzyme activity can be done using non-metabolizing cell lines that have been transfected with cDNA coding for a specific CYP isoform. The drug is incubated with the cells expressing different CYP isoforms and the rate of metabolism in the cells gives information on which enzymes are capable of metabolizing the drug of interest. However, since looking at one enzyme at a time is not an accurate reflection of the *in vivo* situation, these results are usually confirmed using specific inhibition experiments in HLM. HLM are subcellular fractions of liver cells which contain all CYP enzymes. By incubating the drug with HLM and an inhibitor of a specific CYP enzyme, one can draw some conclusions on how much that enzyme contributes to the overall metabolism of the drug.

1.5.2 METABOLITE IDENTIFICATION

Another aspect of understanding the metabolism of a drug is the identification of potential metabolites. It is of great importance to understand if and how any active metabolites are formed as that may impact the safety and efficacy of the drug. Metabolite identification can be performed *in vitro* through analysis of the incubation samples produced from, for example, recombinant enzymes or HLM. Different technologies are available for such analysis. One that is commonly used is High-Resolution Mass Spectrometry (HR-MS) [107]. This technique provides information about unknown compounds through their molecular mass and fragmentation patterns. However, further analysis, for example using nuclear magnetic resonance (NMR), may be required for complete identification of an unknown compound. In addition, *in vivo* experiments are naturally needed to confirm *in vitro* findings.

1.6 PHARMACOMETRICS

Pharmacometrics is a field of studies that uses mathematical models to quantify drug and disease mechanisms in order to optimize drug development and drug treatments. Modeling of the pharmacokinetics and pharmacodynamics of a drug forms the basis of pharmacometric analysis. The overall aim is to guide and support decisions regarding for example dose selection and study design. Having reliable models can aid in making the drug development process more efficient and thereby reduce both costs and timelines.

1.6.1 NONLINEAR MIXED EFFECTS MODELING

Nonlinear mixed effects modeling is commonly applied within the field of pharmacometrics to model the pharmacokinetics and pharmacodynamics in a population. Nonlinear mixed effects modeling combines fixed effects and random effects, where fixed effects are population parameters that are the same for all individuals and samples, whereas random effects are parameters associated with each individual or sample in a population [108]. Using nonlinear mixed effects modeling, one can thereby quantify the amount of variability in the studied population. These models also allow the inclusion of covariates that may explain part of the observed variability. In pharmacokinetics, common covariates that may impact the observed data are, for example, body weight or different genetic polymorphisms in drug metabolizing enzymes. One of the main advantages of using nonlinear mixed effects modeling is that data from the whole population can be analyzed simultaneously. This allows for analysis of data from both sparse and dense sampling.

Using nonlinear mixed effects modeling, a dependent variable y, for the i:th individual and the j:th observation, can be described as a function of independent variables x (e.g. time), fixed effects (θ), random effects for the i:th individual (η_i) and covariates (X_i) as

$$y_{i,j} = f(x, \theta, \eta_i, X_i) + \varepsilon_{i,j} \quad (1)$$

where ε is the residual variability describing the difference between the model prediction and the observed value.

Nonlinear mixed effects modeling applied to pharmacokinetic data is commonly referred to as population pharmacokinetics. A population pharmacokinetic model can be divided into two parts, a structural model and a statistical model. Consider for example a drug exhibiting a mono-exponential decline in plasma concentrations over time after intravenous administration, i.e., the simplest form of a pharmacokinetic model. This can be described by the following equation.

$$C_{p,t} = \frac{Dose}{V} * e^{-\frac{CL}{V}*t} \quad (2)$$

Where $C_{p,t}$ is the plasma concentration at time t, V is the volume of distribution and CL is the clearance. This is the structural model, including only the fixed effects (thetas), which in this case are clearance and volume.

The statistical part of the model describes the variability in the data. This variability can be divided into three different categories; interindividual variability, inter-occasion variability and residual variability.

Interindividual and inter-occasion variability describes the size of the variability between different subjects and occasions. This variability is described by a vector $\eta_{i,n}$, containing all the etas for an individual i, where n is the number of elements in the vector. The etas are commonly assumed to be normally distributed with a mean of zero and a variance of ω^2. In population pharmacokinetic modeling, the population level random parameter that is estimated is usually the variance, ω^2. In Equation 2, inter-individual variability could be incorporated in the clearance and/or volume parameters. This is commonly done assuming an exponential relationship, making the parameters log normally distributed. For each individual i, CL_i and V_i could then be estimated as

$$CL_i = CL_{pop} * e^{\eta_1} \quad (3)$$

$$V_i = V_{pop} * e^{\eta_2} \quad (4)$$

where CL_{pop} and V_{pop} are the theta values for CL and V, i.e. the typical values in the population. Variability between individuals naturally occurs due to biological differences. This variability can sometimes be explained by

recorded covariates in the population. Variability between occasions can, for example, occur in parameters such as absorption due to differences in food intake between different administrations.

Residual variability is the unexplained difference between the individual prediction and the observation. This difference may, for example, be caused by analytical error or inaccurate recording of the time when the plasma sample was taken. For each individual i and observation j, the residual variability is described by an epsilon value $\varepsilon_{i,j}$. The residual variability is estimated as the variance of all the epsilon values, σ^2.

During the model development process, different structural and statistical models are evaluated to find the model that best describes the data. This is an iterative process that requires an understanding of both data analysis and statistics as well as clinical trial design and data collection. A decision on a final model should be based both on statistical goodness-of-fit measures as well as a judgement of the plausibility and appropriateness of the estimated parameters.

The main advantage of having reliable models of the pharmacokinetics and pharmacodynamics of a drug is that they can be used to predict what would happen in other non-studied scenarios. This can be valuable when evaluating, for example, new dose regimens or clinical trial designs before performing a clinical study. Such an approach is a powerful way to support drug development, referred to as model-informed drug development [109-111].

2 AIM

The overall aim of this thesis was to characterize the pharmacokinetics and pharmacodynamics of DMT and to use this information to design new dose regimens and guide future clinical studies.

The specific aims were:

1. To develop and validate a LC-MS/MS method for quantification of DMT and metabolites in human plasma (Paper I)

2. To characterize DMT pharmacokinetics and pharmacodynamics using nonlinear mixed effects modeling (Papers II, III, V and VI)

3. To investigate the metabolic pathways of DMT *in vitro* (Paper IV)

4. To suggest an optimized continuous intravenous infusion protocol for extended administration of DMT (Papers V and VI)

3 METHODS

3.1 CLINICAL STUDIES AND DATA

The clinical data that this thesis is based on comes from two clinical studies performed at Imperial College Research Facility at Imperial College London [16, 19]. Both studies were conducted according to the principles laid down in the revised Declaration of Helsinki (2000), the International Committee on Harmonisation Good Clinical Practices guidelines, and the UK National Health Service Research Governance Framework and were approved by the National Research Ethics Committee London – Brent and the Health Research Authority. All subjects provided written informed consent to participate in the studies.

3.1.1 PILOT STUDY

In the first study [16], DMT was administered as an intravenous bolus dose to 13 healthy subjects. Each subject received placebo as well as one out of four different DMT fumarate doses (7, 14, 18 or 20 mg) on two separate occasions in a single blind, fixed-order fashion. Approximately nine blood samples per subject and occasion were collected pre-dose as well as at staggered time points up to 60 minutes after drug administration. Samples were centrifuged, plasma was harvested and stored at -80°C before analysis. The intensity of the psychedelic experience was assessed by asking participants to rate the intensity of the experience on a scale from 0-10, where 0 corresponded to no psychedelic experience, and 10 was the most intense psychedelic experience imaginable. Ratings were obtained pre-dose as well as every minute during the first 20 minutes after drug administration. EEG recordings were also obtained during the first 20 minutes after administration. The data were divided into the following frequency bands: delta (1–4 Hz), theta (4–8 Hz), alpha (8–13 Hz), beta (13–30 Hz) and low gamma (30–45 Hz). Spontaneous signal diversity was computed to obtain a score of Lempel Ziv-complexity. Data were summarized as mean values per minute within each individual for modeling purposes.

3.1.2 INFUSION STUDY

In the second study [19], DMT was administered as an intravenous bolus dose followed by a continuous infusion over 30 minutes. The first part of the study included eleven healthy subjects. Each subject received placebo as well as two to four doses of DMT fumarate (1.7 mg + 0.2 mg/min [1 subject], 6 mg + 0.6 mg/min [6 subjects], 10 mg + 1.0 mg/min [10 subjects], 14 mg + 1.5 mg/min [9 subjects], and 18 mg + 1.9 mg/min [6 subjects]). In the second part of the

study, 20 healthy subjects received placebo as well as one high dose combination of DMT fumarate (20 mg + 2.27 mg/min) based on the results from the first part of the study. Blood samples were collected pre-dose as well as at 2, 5, 10, 20, 29, 32, 37, 40, 50, 60, 80, 100, 120, 150 and 180 minutes after start of drug administration. Plasma was harvested and stored at -80°C before analysis. Subjective intensity ratings were collected pre-dose as well as at times 1, 2, 3, 4 minutes and then every second minute up to 52 minutes after drug administration in the first part of the study and at times 1, 2, 3, 4, 6, 10 minutes and then every four minutes up to 54 minutes after drug administration in the second part. EEG recordings were also obtained in this study. However, these data were not analyzed as part of this thesis.

3.2 BIOANALYSIS

Plasma samples as well as *in vitro* metabolism samples were quantified for DMT, IAA and DMT-NO using a LC-MS/MS method (Paper I).

3.2.1 INSTRUMENTATION

The LC system consisted of two PE-200 LC pumps (Perkin Elmer, Waltham, MA, USA) connected to a PAL HTC autosampler (CTC Analytics AG, Zwingen, Switzerland). Detection was achieved with an API 4000 triple quadrupole mass spectrometer (AB Sciex, Framingham, MA, USA) operated in positive ion mode using multiple reaction monitoring. Source, curtain and exhaust gas were obtained using an ABN2ZA LC-MS nitrogen gas generator (Peak Scientific Instruments Ltd, Inchinnan, UK).

3.2.2 CHROMATOGRAPHIC CONDITIONS

Analytes were separated on a SpeedCore Diphenyl Column (50 x 2.1 mm, 2.6 µm, Fortis Technologies Ltd, Cheshire UK). Gradient elution was employed using water with 0.1% formic acid as mobile phase A and methanol with 0.1% formic acid as mobile phase B. After an initial two-minute hold at 5%, mobile phase B was linearly increased to 95% over one minute. This was followed by a two-minute hold at 95%, followed by a linear decrease to 5% mobile phase B and a final re-equilibration step at 5% mobile phase B for two minutes. The mobile phase flow rate was 400 µL/min.

3.2.3 SAMPLE PREPARATION

An aliquot of 200 µL plasma was mixed with 50 µL methanol containing internal standards. This was followed by protein precipitation through addition of 600 µL acetonitrile. Samples were agitated for two minutes and centrifuged

at 13000 x *g* for 15 minutes. Supernatants were transferred to new tubes and evaporated. The residue was reconstituted in 100 µL water containing 0.1% formic acid.

3.2.4 VALIDATION

Method validation was performed according to FDA's Guidance for Industry: Bioanalytical Method Validation [112] with regards to accuracy, precision, sensitivity, selectivity, recovery, matrix effects, stability, carry-over and dilution integrity. For IAA, a background subtraction approach was used [113] as it is known to be present endogenously.

3.3 *IN VITRO* METABOLISM

3.3.1 REACTION PHENOTYPING

The potential involvement and contribution of CYP enzymes in DMT metabolism was investigated using recombinant CYP enzymes and HLMs. DMT was incubated with each CYP isoenzyme and a nicotinamide adenine dinucleotide phosphate (NADPH) regenerating system in phosphate buffer (100 mM, pH 7.4). Incubations were also performed with HLM and an NADPH regenerating system in phosphate buffer in the presence or absence of quinidine (a specific CYP2D6 inhibitor), harmine (inhibitor of MAO-A and CYP2D6) or SKF-525A (a general CYP inhibitor). Incubations were performed at 37°C for 60 minutes and aliquots of 100 µL were removed at 0, 5, 10, 20, 30 and 60 minutes. Reactions were quenched by addition of ice-cold acetonitrile and samples were centrifuged at 10000 × *g* for 2 minutes. Supernatants were transferred into new vials and were either analyzed directly or stored at -80°C before analysis.

DMT half-life was calculated as

$$Half\ life = \frac{\ln(2)}{k} \tag{5}$$

where k is the slope of the logarithm of DMT concentrations plotted over time.

3.3.2 METABOLITE IDENTIFICATION

After incubating DMT with CYP2D6, samples were further analyzed to screen for unidentified metabolites. Briefly, analysis was performed using a Waters Aquity I-class ultra-performance liquid chromatography (UPLC) system coupled to a Vion ion-mobility spectrometry (IMS)-quadrupole time-of-flight tandem mass spectrometer (Waters Corporation) equipped with an electrospray interface operated in positive electrospray ionization mode.

Analytes were separated on a Waters Acquity UPLC HSS T3 analytical column (2.1 x 150 mm, 1.8 µm particle size). An injection volume of 1 µL was used at a flow rate of 0.4 mL/min. Mobile phase A consisted of 5 mM ammonium formate and 0.1% formic acid in water and mobile phase B of 0.1% formic acid in acetonitrile. A linear gradient of 2-15% B over 15 minutes was followed by a second gradient of 15-95% B over 0.5 minutes and ended in isocratic elution at 95% B for 2 minutes. The column was equilibrated at 2% B for 2.2 minutes before the next injection. MS data were acquired using alternating high and low collision energies for collection of full-scan data. The scan range was m/z 50-800 and the scan time was 0.15 s. Product ion scans (MS/MS) were acquired on detected major metabolites. The Waters UNIFI software (v 3.1) was used for data acquisition and processing. The software automatically searched for selected phase I metabolites and cleaved metabolites. All tentative metabolites with a mass accuracy <5ppm and a response over 300 were considered.

3.4 NONLINEAR MIXED EFFECTS MODELING

3.4.1 SOFTWARE

Data were analyzed using NONMEM version 7.4.3 (ICON Development Solutions, Ellicott City, MD, USA) [114]. Pirana (v.3.0.0) and Perl-speaks-NONMEM (v.5.2.6) [115] were used for model automation and diagnostics. R (v 4.1.1) was used for model diagnostics and visualization.

3.4.2 MODELING APPROACH

Models were fitted using either the first-order conditional estimation method with interaction (Papers II, III, V and VI) or the Laplace estimation method (Paper V). All DMT fumarate doses were converted to nanomoles of DMT base for modeling purposes.

Modeling was performed using a sequential approach. A pharmacokinetic model was first developed using data from the pilot study only (Paper II). A model was developed describing DMT plasma concentrations, this model was then extended to also include the metabolite IAA. Once more data became available, the model was further developed, combining all available pharmacokinetic data (Paper VI).

Pharmacokinetic/pharmacodynamic models were developed to describe the relationships between DMT exposure and subjective psychedelic intensity ratings (Papers II, V and VI), alpha waves (Paper III), beta waves (Paper III) and Lempel-Ziv complexity scores (Paper III). All pharmacokinetic/pharmacodynamic models were developed using a

population pharmacokinetic parameter and data approach, where population pharmacokinetic parameters are fixed, but individual pharmacokinetic parameters are estimated simultaneously with pharmacodynamic parameters [116].

A slight delay was observed between DMT plasma concentrations and all assessed pharmacodynamic measurements. This delay was described using effect compartment models where the assumption is that the response is mediated through DMT levels in the biophase, represented by a theoretical effect compartment. The change of concentration in the effect compartment is described according to

$$\frac{dC_e}{dt} = k_{e0} * \left(C_p - C_e\right) \quad (6)$$

where k_{e0} is the effect compartment equilibrium rate constant, C_p is the plasma concentration, and C_e is the theoretical concentration in the effect compartment.

Model discrimination between nested models was based on objective function value (OFV), where a change in OFV of -3.84 was considered a significant model improvement at p=0.05 under the assumption that ΔOFV is approximately χ^2 distributed. Model performance was also assessed using visual predictive checks, goodness-of-fit plots, parameter precision and plausibility of parameter estimates. Parameter precision was determined using sampling importance resampling [117].

3.4.3 PHARMACOKINETIC MODEL

One-, two- and three-compartment pharmacokinetic models with first-order elimination were fitted to DMT observations. IAA plasma concentrations were described using a one-compartment model with first-order elimination. The formation rate of IAA was assumed to be equal to the elimination rate of DMT, using a fixed metabolic fraction of 1. Between subject variability was assessed on all parameters as exponential random effects following a log-normal distribution with mean zero and variance ω^2. Residual variability was evaluated separately for DMT and IAA using additive, proportional or combined error models.

3.4.4 SUBJECTIVE INTENSITY RATINGS

As subjective intensity ratings were obtained on a scale from 0-10, where only integer values are allowed, this is a discrete bounded variable that can be handled in a number of different ways. The relationship between DMT exposure and subjective intensity was initially described by treating the ratings as a continuous variable (Papers II and VI). However, a bounded integer model

[118], respecting both the integer nature of the data and the boundaries of the scale, was also developed to assess how the choice of modeling approach might impact dose decisions for DMT (Paper V).

3.4.4.1 CONTINUOUS VARIABLE MODEL

The relationship between DMT exposure and subjective intensity ratings was assessed using a sigmoid E_{max} model according to

$$Response = E_0 + \frac{Emax * C_e^{\gamma}}{EC50_e^{\gamma} + C_e^{\gamma}} \quad (7)$$

where E_0 is the baseline response, E_{max} is the maximum response, $EC_{50,e}$ is the concentration in the effect compartment needed to produce half of the maximum response, and the Hill coefficient γ describes the sigmoidicity of the relationship.

As the ratings were handled as a continuous variable, a logit transformation was used to restrict predictions between 0 and 10. This was implemented for every observation j of each individual i as

$$y = 10 * \frac{e^{\ln\left(\frac{\lambda}{10-\lambda}\right)+\varepsilon_{ij}}}{1+e^{\ln\left(\frac{\lambda}{10-\lambda}\right)+\varepsilon_{ij}}} \quad (8)$$

where λ is the individual prediction, and ε_{ij} is the residual error, additive on the logit scale and following a normal distribution.

Between subject variability was assessed on k_{e0}, $EC_{50,e}$ and γ.

3.4.4.2 BOUNDED INTEGER MODEL

This model treats the data as a discrete variable and, consequently, models the probability of observing a particular score rather than modeling the scores as such [118].

As the subjective rating scale consists of eleven categories, the area under the standard normal distribution was divided into eleven (n) equally sized areas. The probit function was used to define ten cut-off values ($Z_{1/n}$- $Z_{(n-1)/n}$). A function describing the mean of a normal distribution, using fixed effects (θ), random effects for an individual i (η_i), time and covariates (X_i), $f(\theta,\eta_{i,f},t,X_{i,f})$, together with a function describing the variance of a normal distribution, using fixed effects (σ), random effects for an individual i (η_i), time and covariates (X_i), $g(\sigma,\eta_{i,g},t,X_{i,g})$, were used along with the cut-off values to estimate the probability of each score. The probability for the *k*th score is

$$P_{i,j}(k) = \Phi\left(\frac{Z_{\frac{k}{n}} - f(\theta,\eta_{i,f},t,X_{i,f})}{g(\sigma,\eta_{i,g},t,X_{i,g})}\right) - \Phi\left(\frac{Z_{\frac{k-1}{n}} - f(\theta,\eta_{i,f},t,X_{i,f})}{g(\sigma,\eta_{i,g},t,X_{i,g})}\right) \quad (9)$$

where Φ is the cumulative distribution of the normal distribution function.

For the first category (k=1) this collapses into

$$P_{i,j}(1) = \Phi\left(\frac{Z_{\frac{1}{n}} - f(\theta,\eta_{i,f},t,X_{i,f})}{g(\sigma,\eta_{i,g},t,X_{i,g})}\right) \quad (10)$$

and for the last category (k=n) into

$$P_{i,j}(n) = 1 - \Phi\left(\frac{Z_{\frac{n-1}{n}} - f(\theta,\eta_{i,f},t,X_{i,f})}{g(\sigma,\eta_{i,g},t,X_{i,g})}\right) \quad (11)$$

representing the cumulative distribution within the intervals ($-\infty$, $Z_{1/n}$) and ($Z_{(n-1)/n}$, ∞), respectively.

The relationship between DMT exposure and the individual prediction of the mean of the normal distribution was described by a linear function. Between subject variability was assessed on drug effect and variance.

Additionally, serial correlation in the data was assessed through the implementation of a Markov element [118] according to

$$P_{i,j}(k|Y_{i,j-1} = k) = \frac{P_{k,i,j}+PM}{1+PM} \quad (12)$$

where $Y_{i,j-1}$ is the previous observation and $P_{k,i,j}$ is the probability of a score k for individual i at time j.

If $Y_{i,j}$ and $Y_{i,j-1}$ are different, the equation simplifies to

$$P_{i,j}(k|Y_{i,j-1} \neq k) = \frac{P_{k,i,j}}{1+PM} \quad (13)$$

A positive value of the Markov parameter PM is associated with a higher probability of an observation having the same value as the previous observation.

3.4.5 EEG RECORDINGS

All the available EEG data were visually explored for any potential exposure-response relationship. However, apparent exposure-response relationships could be distinguished only for alpha power, beta power and Lempel-Ziv complexity. Hence, any effects in delta, theta and gamma power were not modeled as part of this thesis.

The relationships between DMT exposure and alpha power, as well as beta power, were described by inhibitory sigmoid I_{max} models according to

$$Response = R_0 * (1 - \frac{Imax*C_e^\gamma}{IC50_e^\gamma + C_e^\gamma}) \quad (14)$$

where R_0 is the baseline response, i.e., the alpha or beta power in absence of drug, I_{max} is the maximum decrease in alpha or beta power, $IC_{50,e}$ is the concentration of DMT in the effect compartment required to produce half of the maximum response and γ is a slope factor describing the sigmoidicity of the relationship.

The effect on Lempel-Ziv complexity was described by a sigmoid E_{max} model according to

$$Response = R_0 * (1 + \frac{Emax * C_e^{\gamma}}{EC50_e^{\gamma} + C_e^{\gamma}}) \quad (15)$$

where R_0 is the Lempel-Ziv complexity score in the absence of drug, E_{max} is the maximum increase in Lempel-Ziv complexity score and $EC_{50,e}$ is the concentration of DMT in the effect compartment required to produce half of the maximum response, and γ is a slope factor describing the sigmoidicity of the relationship.

Between subject variability was assessed on all parameters. Between occasion variability was evaluated on baseline response. A Box-Cox transformation of the between subject variability in the baseline alpha power was performed to account for a skewed distribution. Residual variability was assessed as additive, proportional or combined errors.

3.5 SIMULATIONS

3.5.1 EXPECTED RESPONSE AT DIFFERENT INTRAVENOUS BOLUS DOSES

Simulations were performed to demonstrate the expected exposure, effect and variability associated with different dose levels of DMT administered as different intravenous bolus doses (Papers II and III). These simulations were performed in R using the package mrgsolve and did not include residual variability. Simulations were performed in 100 subjects across a range of doses that were expected to cause non-existent to significant psychedelic effects.

3.5.2 DOSE RECOMMENDATIONS FOR INTRAVENOUS INFUSION PROTOCOL

Simulations were performed with the pharmacokinetic/pharmacodynamic models describing the relationship between DMT exposure and subjective intensity ratings with the aim of identifying a combination of an intravenous bolus dose and an infusion rate that would keep participants at a target intensity rating over a longer period of time (Paper V). Simulations were performed in

NONMEM using both between subject variability and residual variability. Subjective intensity ratings were simulated in 1000 subjects at two minutes after administration of a bolus dose as well as at steady-state. The proportions of the population within, below or above a target of ratings between 7-9 or 4-6 were assessed.

3.5.3 INDIVIDUALIZED DOSING OF DMT

To design an infusion schedule that would allow for individual adjustments based on observed intensity ratings, simulations were performed in a virtual population of 1000 subjects using package mrgsolve for R (Paper VI). Simulations included between subject variability but no residual variability. In a first step, the aim was to find a starting dose that would give the highest proportion of the simulated population within a target of ratings between 8-9 at steady-state without having more than 20% of the population exceeding the target. The simulated population was then divided into six different sub-populations depending on the predicted response – ratings 0-1, ratings 2-3, ratings 4-5 ratings 6-7, ratings 8-9 and ratings >9. For the subpopulations below or above target, the simulation step was repeated to find a dose that would lead to the highest proportion of these subpopulations within target. This was then repeated twice for those subjects still below or above target. If there was a less than 3% difference in the predicted proportion within target between two doses, the lowest dose was chosen for safety reasons. For the same reason, for subpopulations below target, only dose changes leading to less than 10% of the population above target were considered. Based on these simulations, a protocol for how to adjust the dose based on observed intensity ratings was developed.

4 RESULTS AND DISCUSSION

4.1 LC-MS/MS METHOD FOR ANALYSIS OF DMT AND METABOLITES (PAPER I)

A LC-MS/MS method was developed for the quantification of DMT, IAA and DMT-NO in plasma. The final method included sample preparation through protein precipitation, followed by a drying step and reconstitution in water with 0.1% formic acid. Other sample preparation procedures were also evaluated, including liquid-liquid extraction with various solvents as well as a salting out liquid extraction procedure. However, none was successful in sufficiently extracting all three analytes. It was also observed that DMT was chemically converted to DMT-NO in the presence of certain solvents. Protein precipitation with acetonitrile was therefore considered a suitable solvent to avoid such conversion. Separation was achieved using gradient elution on a SpeedCore Diphenyl column with a total run time of 7 minutes. This column was chosen over a C18 column to overcome issues with ion suppression. Overall, the short run time and simple sample preparation procedure make this method efficient and easily applied to a larger number of samples.

Validation was performed according to FDA guidelines [112]. The method was accurate and precise over concentrations of 0.25-200 nM for DMT, 15-250 nM for DMT-NO and 500-5000 nM for IAA. Example chromatograms at the lower limits of quantification as well as in blank plasma are shown in Figure 5. Selectivity could not be demonstrated for IAA as it is present in large amounts in untreated individuals. In addition, some blank plasma samples contained small peaks corresponding to the fragmentation and retention time of DMT. It is possible that these peaks correspond to endogenous DMT. However, any potential impact of these peaks on quantification was considered negligible. Furthermore, as IAA is present endogenously, no absolute lower limit of quantification could be set. Rather, a background subtraction approach was used and a change in response that could be differentiated from that in the blank was used as a surrogate marker of the lower limit of quantification [113].

Relative recovery ranged from 63.5-97.4% across the different concentrations of the different analytes. A significant matrix effect was also observed for DMT-NO. However, both recovery and matrix effects were consistent within each concentration level and were not considered to affect the quantification.

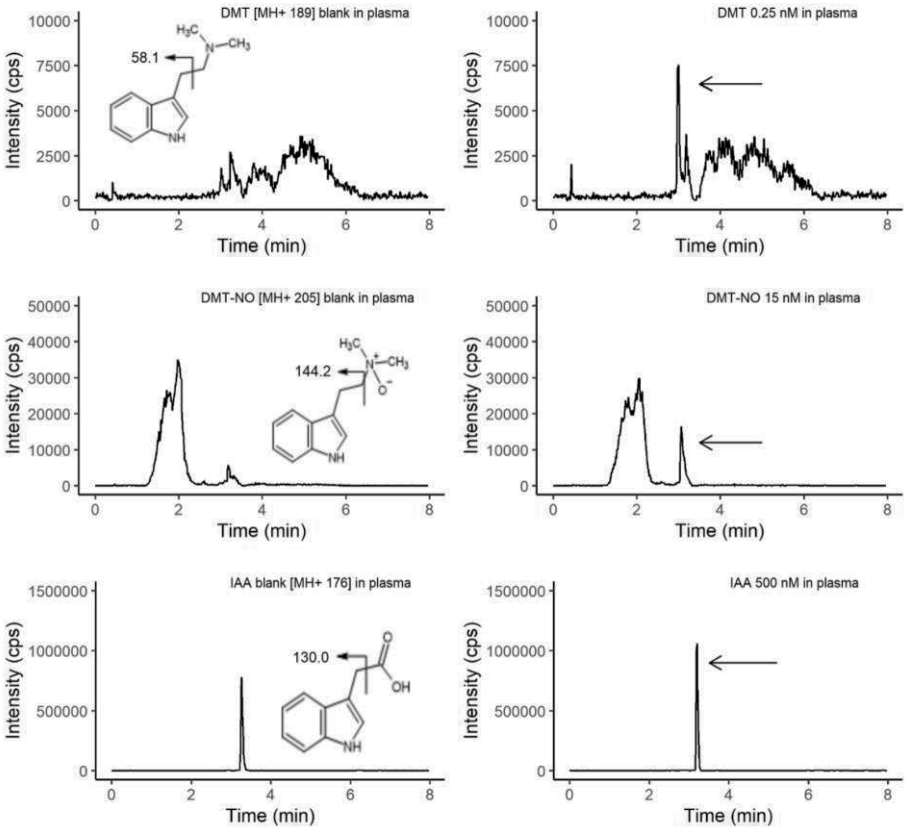


Figure 5. Chromatograms, molecular structures and suggested fragmentations of DMT, DMT-NO and IAA in blank plasma samples (left panels) and at their lower limit of quantification levels (right panels). Reprinted with permission from Journal of Pharmaceutical and Biomedical Analysis:[119]

Samples were shown to be stable after 4 hours on the bench top, 24 hours in the autosampler, 3 freeze-thaw cycles and after 3 months in -80°C. The analytes were also shown to be stable in whole blood for up to 60 minutes. The samples can consequently be considered stable under the applied storage and preparation conditions.

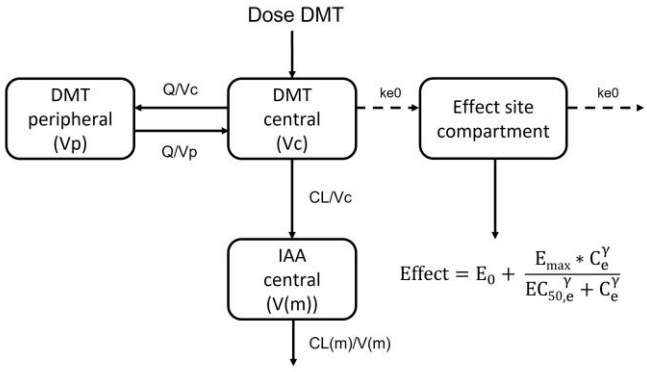
The applicability of the method was demonstrated through the analysis of clinical samples. This method serves as the basis for this entire thesis, as no pharmacometric analysis could have been performed without an accurate and precise bioanalytical method to obtain reliable data.

4.2 PHARMACOKINETICS AND SUBJECTIVE PSYCHEDELIC INTENSITY (PAPER II)

This work aimed to characterize the population pharmacokinetics of DMT as well as the relationship between DMT exposure and its subjective psychedelic effects. This was done using data from the pilot study described in section 3.1.1. Data were analyzed using nonlinear mixed effects modeling as described in section 3.4. The subjective intensity scores were treated as continuous data.

DMT plasma concentrations were described by a two-compartment model with first-order elimination, leading to the formation of the metabolite IAA via a single elimination pathway. IAA concentrations were described by a one-compartment model with first-order elimination. Between subject variability was incorporated on DMT clearance and IAA volume of distribution. The relationship between DMT concentrations and the subjective psychedelic intensity ratings were described by an effect compartment model with a sigmoid E_{max} response (Equation 7). Between subject variability was incorporated on $EC_{50,e}$ and the Hill factor γ.

The reader is referred to Paper II for an extensive description of the model development and the final parameter estimates. A schematic of the model with some of the estimated population parameters is depicted in Figure 6 and the following should be noted: Firstly, in this work, the elimination of DMT was described with a single elimination pathway leading to the formation of the metabolite IAA. This approach was chosen as IAA is known to be the major metabolite of DMT, and no other data were available. Our analysis showed that no quantifiable levels of DMT-NO were present in these samples, but there may be other metabolites present that have not been accounted for. Consequently, this may not be an accurate reflection of the full pharmacokinetic fate of DMT. Nevertheless, this was deemed the most appropriate option with the data at hand and the model could potentially be revised in the future with more information on the metabolic pattern of DMT.



Parameter	Estimate
CL	26.0 L/min
Q	2.99 L/min
Vc	221 L
Vp	59 L
CL(m)	0.093 L/min
V(m)	9.55 L
E_{max}	10
$EC_{50,e}$	94.7 nM
γ	2.87

Figure 6. Schematic of final model describing the relationship between DMT exposure and subjective psychedelic intensity.

Secondly, the estimated clearance of DMT was 26 L/min. This is an extremely high value, indicating that DMT elimination is independent of blood flow. Drugs that are eliminated via metabolism are usually restricted to a maximum clearance of 1.5 L/min, corresponding to the hepatic blood flow rate. This is because most drug metabolizing enzymes reside in the liver. However, as MAO-A is present, not only in the liver, but throughout the entire body [120, 121], this high clearance value is likely a reflection of that. This is also the main reason for the short half-life of DMT, estimated here at approximately 7 minutes.

Further, a high degree of variability was observed in several pharmacokinetic and pharmacodynamic parameters. This means that the obtained exposure to DMT, as well as the observed effects, may vary substantially between individuals after administration of identical DMT doses. This is illustrated in Figure 7, which depicts the predicted response across different intravenous bolus doses in a simulated population of 100 individuals (section 3.5.1). There are several potential explanations for this, including biological factors such as weight and polymorphisms in metabolic enzymes leading to different people having different capacities of eliminating DMT. Another explanation to the variability in response is the subjective nature of the response measurement. While all participants in this study have previous experience with psychedelics, and hence some reference point as to what would be considered an intense psychedelic experience, the difference between, for example, rating 5 and 7 on this rating scale is not clear-cut. It is evident that more research is needed to investigate the underlying causes of the observed variability.

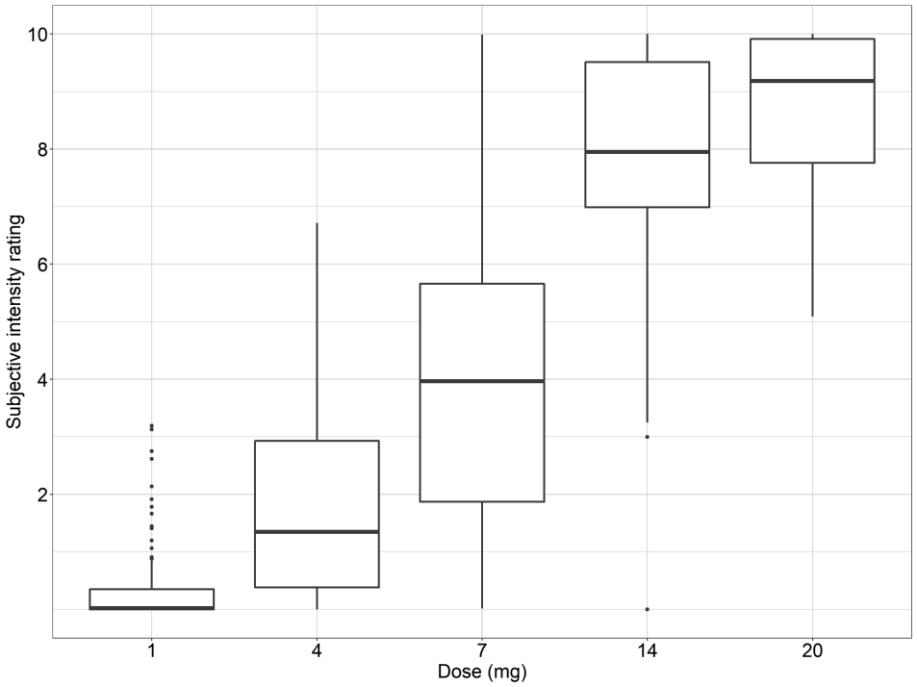


Figure 7. Distribution of simulated maximum achieved effect at five different intravenously administrated DMT fumarate bolus doses in 100 individuals, respectively. The horizontal line in the middle of the boxes is the median, the boxes indicate the 25th and the 75th percentiles and whiskers extend between the 5th and the 95th percentiles. Reprinted with permission from Clinical and Translational Science:[122]

The main limitation of this study is the small study sample (n=13). However, it is an important first step into describing the pharmacokinetics and pharmacodynamics of DMT and, to the best of our knowledge, the first published population pharmacokinetic analysis of DMT data.

4.3 EEG EFFECTS (PAPER III)

The aim of this work was to describe the relationship between DMT exposure and the observed effects in alpha power, beta power and Lempel-Ziv complexity score measured by EEG. This was done using data from the pilot study described in section 3.1.1. Data were analyzed using nonlinear mixed effects modeling as described in section 3.4 where DMT had an inhibitory effect on alpha- and beta power, described by inhibitory I_{max} models (Equation 14). The effect on Lempel-Ziv complexity score was stimulatory and described by an E_{max} model (Equation 15).

DMT was shown to fully suppress alpha power and I_{max} was therefore fixed to 1, with an estimated $IC_{50,e}$ of 71 nM. Between subject variability was incorporated in baseline response and $IC_{50,e}$. Between occasion variability was also incorporated in the baseline response as it varied substantially in some participants between the placebo session and the active DMT session. It was also observed that the participants who received the highest DMT dose in general had a higher baseline response. This could potentially impact the obtained parameter estimates, and studies should be performed in a larger number of participants to increase the confidence in the presented model.

DMT partially inhibited beta power with an estimated I_{max} of 0.7 (i.e. a 70% decrease from baseline) and an $IC_{50,e}$ of 137 nM, indicating that higher concentrations are needed for suppression of beta power compared to alpha power. Between subject variability was incorporated in baseline response and $IC_{50,e}$. Between occasion variability was incorporated in baseline response for the same reasons as for alpha power. The estimated variability in $IC_{50,e}$ was 75% which is large and, considering the small number of participants, one should be cautious about extrapolating these results to a larger population. Furthermore, the data give reason to believe that the true I_{max} may not have been reached in this study as the DMT effect on beta power did not fully plateau at the highest concentrations. Further research with a higher number of participants, and potentially even higher doses, are needed to increase our understanding of this relationship.

Lempel-Ziv complexity scores were increased after administration of DMT with E_{max} estimated at 0.10 (i.e. a 10% increase from baseline) and $EC_{50,e}$ at 54 nM. A substantial degree of variability was observed also for these parameters with between subject variability incorporated in baseline response, $EC_{50,e}$ and E_{max} and between occasions variability incorporated in baseline response. Again, we cannot be sure that the true maximum response has been reached and more research is needed with more participants and higher doses.

Overall, while there is uncertainty in the obtained parameter estimates, a relationship between DMT exposure and these response measurements does appear to exist. As can be seen in Figure 8, showing the predicted response associated with different intravenous bolus doses of DMT (section 3.5.1), the strongest relationship was that between DMT concentrations and alpha power. This is in agreement with previous research indicating that suppression of alpha power is the most robust EEG effect of psychedelics [26, 42-46]. Lempel-Ziv complexity score has also been stated to be a robust effect of DMT [16, 41], although perhaps not as well studied as alpha power. It is worth noting that the $EC_{50,e}$ value for the increase in Lempel-Ziv complexity score is similar to the $IC_{50,e}$ value describing the potency to reduce alpha power. As the rest of the model parameters are also similar, Lempel-Ziv complexity scores are likely

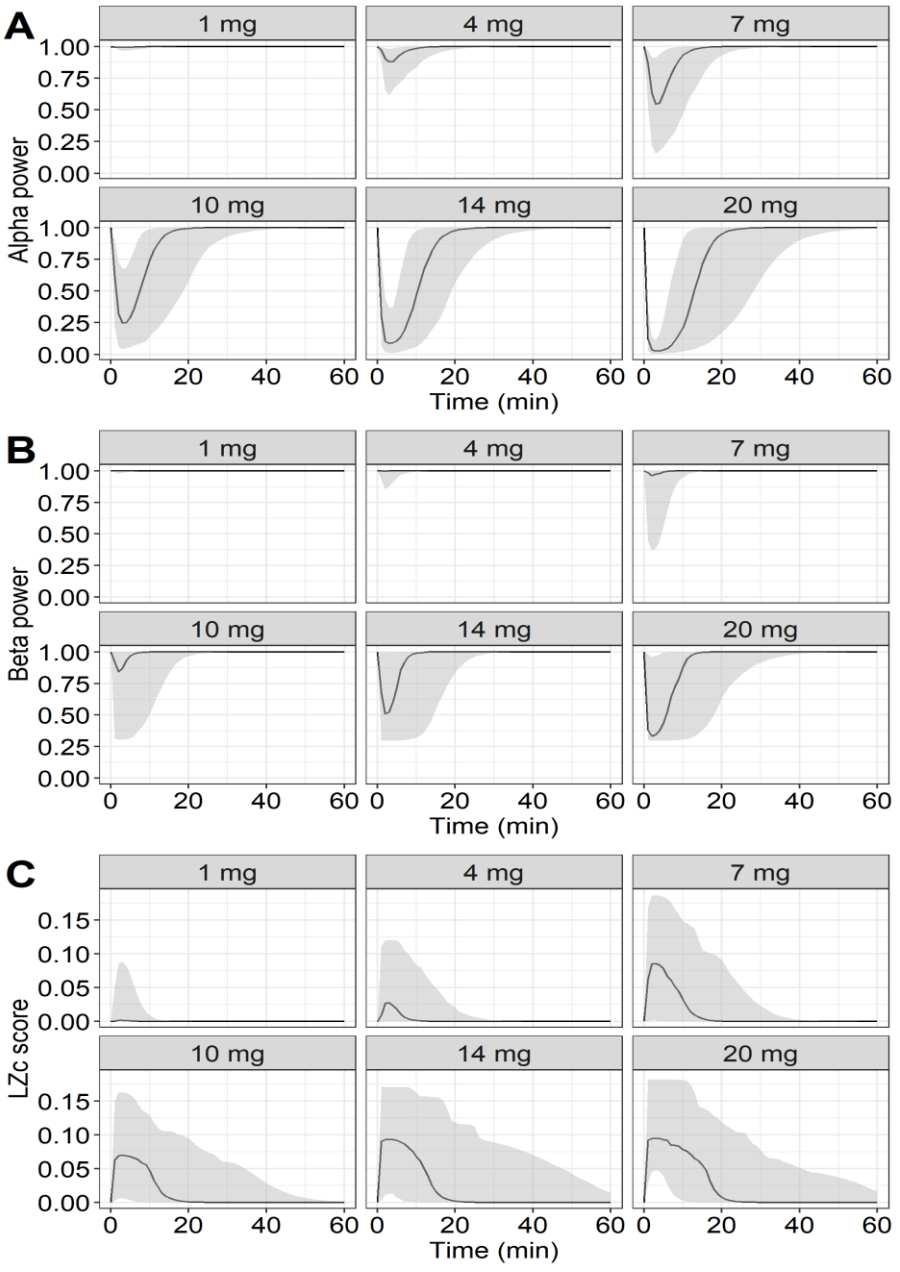


Figure 8. Simulations of relative change in alpha power, beta power and Lempel-Ziv complexity score over time after intravenous bolus administration of DMT fumarate at five different dose levels in 100 individuals. The solid line is the median prediction and the shaded area is the 90% prediction interval. Reprinted with permission from CPT:Pharmacometrics and Systems Pharmacology:[123]

to follow the effects of alpha power, although the variability may be larger between individuals. With beta power on the other hand, the large variability between individuals and the uncertainty in the I_{max} estimate makes it difficult to predict what response to expect at a certain dose. Hence, based on these results, alpha power and Lempel-Ziv complexity scores are more likely to serve as useful endpoints for measuring DMT effects as compared to beta power.

Similar to the previously described analysis of subjective intensity ratings, the main limitation of this work is the limited number of participants. Although a large degree of variability was observed, no underlying covariates could be investigated and this is something that should be evaluated in the future. Nevertheless, this is the first study describing the relationship between DMT exposure and EEG effects, leading to an increased understanding of DMTs effects on the brain. As there is research indicating a relationship between both alpha power and Lempel-Ziv complexity score and depressive symptoms [124-126], it is possible that these could serve as potential biomarkers of the therapeutic effects of DMT in the future. However, more research is needed to understand the relationship between DMT exposure and these EEG markers as well as any potential relationship between EEG effects and therapeutic effects.

4.4 *IN VITRO* METABOLISM (PAPER IV)

The aim of this work was to investigate the CYP mediated metabolism of DMT *in vitro* and to identify which metabolites are formed via this pathway. This was done using the methodology described in section 3.3.

After incubation with a battery of recombinant CYP enzymes, DMT was shown to be a substrate for CYP2D6, which is in agreement with a previously published paper by Good *et al* [20]. However, they also observed metabolism by CYP2C19, whereas in our incubations, DMT was stable with all other investigated enzymes (including CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2E1, CYP3A4 and CYP3A5). In incubations with HLMs, DMT had a half-life of approximately 10 minutes. This half-life was increased after inclusion of the general CYP inhibitor SKF-525A and metabolism was completely inhibited after inclusion of harmine, an inhibitor of MAO-A and CYP2D6. However, inclusion of the CYP2D6 inhibitor quinidine did not have any effect on DMT half-life in HLMs. As significant amounts of IAA were observed in these incubations, it can be concluded that MAO-A was present in the HLMs and that this affected the results. In order to be able to study CYP metabolism in isolation in HLMs, a specific MAO-A inhibitor would be needed. However, we have been unable to identify such a

compound as it appears that some of the known MAO-A inhibitors may also affect CYP mediated metabolism [127].

Samples resulting from incubations with CYP2D6 were further analyzed using HR-MS to identify what metabolites were being formed via this pathway. Four major metabolites were observed, resulting from mono-, di- and tri-oxygenation of DMT. Interestingly, the metabolic pattern changed over time, indicating a stepwise metabolism. Based on the fragmentation patterns, it was concluded that the observed metabolites were most likely a result of hydroxylation on the indole core. A similar metabolic pattern has been proposed for several other di-alkylated tryptamines [128-132], further strengthening this hypothesis. The reader is referred to Paper IV for a depiction of the suggested metabolic pathway.

No final conclusions on the exact positions of the oxygenations can be drawn from our results. However, mono-hydroxylation on the 4- and 5-position can be excluded as these transformations correspond to the known psychoactive compounds psilocin and bufotenine, which were included as reference standards. Consequently, the mono-oxygenated metabolite most likely stems from hydroxylation on the 6- or 7-position of the indole core. As both psilocin and bufotenine are known to be active, it is possible that this metabolite may exhibit similar activity. Further research should focus on the elucidation of the structure of these metabolites and any potential pharmacological activity. This is essential in understanding the risk for any future drug-drug interactions and the potential of DMT as a therapeutic option.

4.5 DESIGNING AN INFUSION PROTOCOL FOR DMT (PAPER V)

The aim of this work was to design an infusion protocol for DMT as well as to investigate the impact of choosing between two different modeling approaches on dose selection. Recommendations for dose selections were made based on a target psychedelic intensity using either the model described in Paper II (sections 3.4.4.1 and 4.2), treating the intensity ratings as a continuous variable, or using a bounded integer model (section 3.4.4.2), treating the data as an ordered categorical variable.

Two different bounded integer models were included here, with and without a Markov element accounting for serial correlation in the data. Adding a Markov parameter led to a significant decrease in OFV but as the continuous variable model did not contain a similar parameter, both models were kept for comparison. The bounded integer models included a linear relationship between drug concentration and effect with a slope estimated at 0.0159 and 0.0163, respectively. The standard deviation of the variance function was

estimated at 0.269 and 0.275, respectively. Between subject variability was incorporated on both drug effect and the variance function. Overall, all three models provided a similar fit to the data based on visual predictive checks, the number of parameters as well as parameter precision were also similar between models.

Simulations were performed with all three models to identify a combination of a bolus dose and an infusion rate that would keep participants at a target intensity rating over a longer period of time (section 3.5.2). Two targets were assessed here. The primary target was set at ratings between 7 and 9, this was to assure a significant psychedelic experience while aiming to avoid a too intense experience, potentially increasing the risk for adverse psychological reactions. A secondary target of ratings between 4 and 6 was also assessed for the purpose of comparing the different models. The median ratings and 90% prediction intervals associated with different dose levels are depicted in Figure 9, and the proportions of the population within, below or above target at steady-state are summarized in Figure 10. Based on the continuous variable model, the highest proportion within a target of 7-9 was predicted to be achieved with a bolus dose of 16 mg followed by an infusion rate of 1.4 mg/min. The corresponding doses for the bounded integer models were 14 mg followed by an infusion rate of 1.2 mg/min. However, variability between individuals was high, and regardless of model choice, the predicted proportions within target were low. At a target of ratings between 4-6, the predicted optimal doses were a bolus dose of 10 mg followed by an infusion rate of 0.8 mg/min with all three models. Also for this target, the predicted proportions within target were low. It was concluded that individually adjusted doses will likely be necessary to achieve target ratings in a larger proportion of the population.

Some key differences were observed between the two modeling approaches. While the predicted proportions within target were low in all cases, they were higher with the continuous variable model as compared to the bounded integer models. At the predicted optimal infusion rates for targets between 7-9, 45% of the population were predicted to be within target based on the continuous variable model, whereas the corresponding proportion was only around 25% based on the two bounded integer models. This was mainly caused by a higher proportion ending up above target with the bounded integer models as compared to the continuous variable model. This can also be seen at higher dose levels where the continuous variable model predicts median ratings within target at infusion rates up to 2.6 mg/min, whereas the bounded integer models predict median ratings above target already at doses above 1.4 mg/min. From a safety perspective, this difference could have a major impact on dose decision-making, as doses that might be considered unsafe with the bounded integer model would be considered safe based on the continuous variable model.

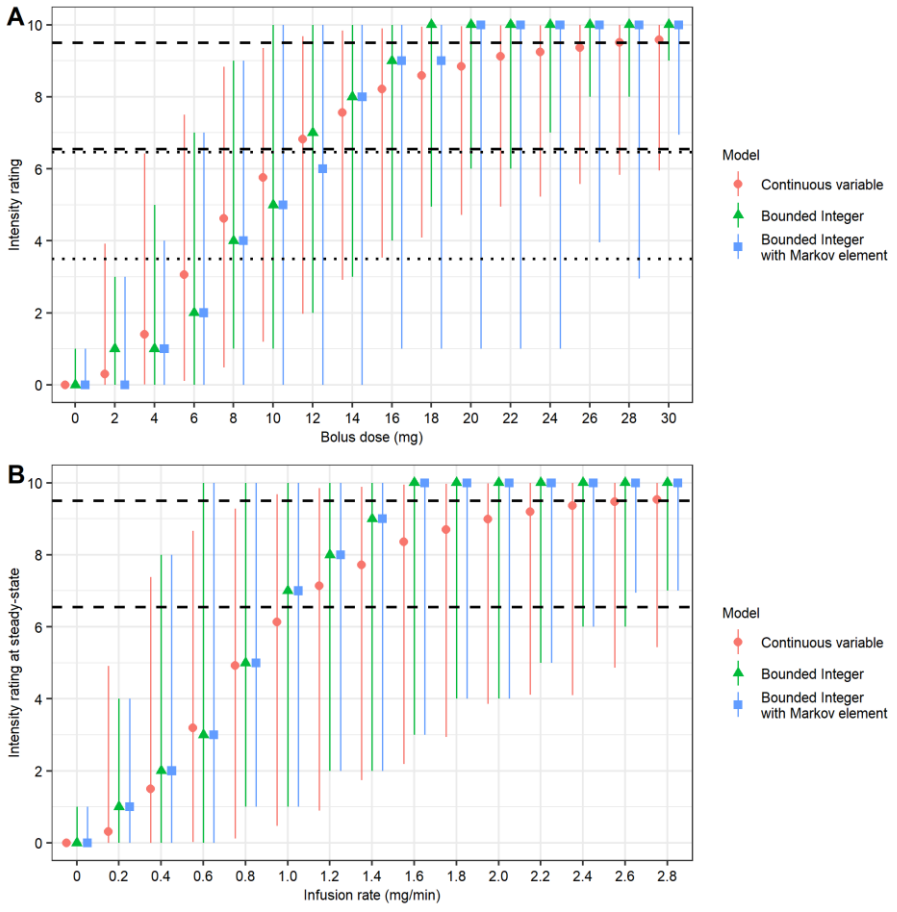


Figure 9. Predicted distribution of intensity ratings at A) two minutes after a DMT bolus dose across different dose levels and B) at steady-state across different infusion rates. Solid lines represent the 90% prediction interval at each dose level with filled shapes representing the median prediction. Dashed lines demonstrate a target response interval of 7-9 and the dotted line represent a target response interval of 4-6. Reprinted with permission from CPT:Pharmacometrics and Systems Pharmacology:[133]

There are a number of limitations to this study. The limited number of study participants is one, and the fact that we cannot conclude from this work if the recommended doses are clinically suitable or not is another. In addition, the purpose of this work was not to make the models comparable as such and it is possible that this may have impacted the results. Nevertheless, we believe the provided dose recommendations serve as a good starting point for researchers aiming to administer DMT as a continuous infusion. However, individualized

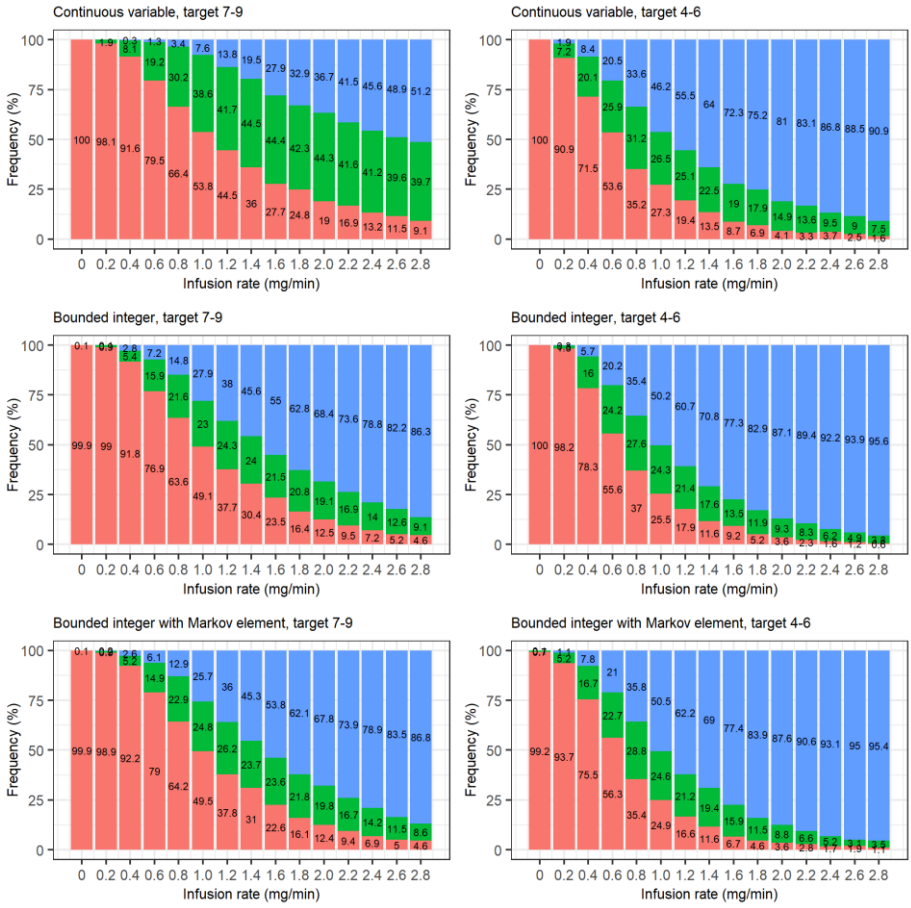


Figure 10. Predicted proportions of the population having an intensity rating within target (green), below target (red) and above target (blue) at steady-state across different DMT infusion rates. Panel A demonstrates predicted proportions at a target response of ratings between 7-9. Panel B demonstrates predicted proportions at a target response of ratings between 4-6. Reprinted with permission from CPT:Pharmacometrics and Systems Pharmacology:[133]

dosing will likely be necessary and more research is needed to determine factors that could guide such dosing. Furthermore, it appears that the impact of choosing either a continuous variable model or a bounded integer model will depend on the target and the purpose of the model. The main differences lie at the boundaries of the scale and while the recommended doses in this case are similar regardless of model choice, there may be other cases where these differences could have a significant impact on dose decisions.

4.6 A FRAMEWORK FOR INDIVIDUALIZED INFUSION RATES (PAPER VI)

The aim of this work was to further develop the already available pharmacokinetic/pharmacodynamic model described in Paper II as well as to use this model to demonstrate how a better understanding of the pharmacokinetic/pharmacodynamic relationship for DMT can guide dose decisions in the future. The data obtained in the infusion study described in section 3.1.2 was pooled with data from the pilot study (section 3.1.1). This was done in two steps: 1) Data from the first dose finding part of the study [19] was first added and this intermediate model was used to guide dosing in the second part of the study and 2) once data from the second part was available, the model was further developed and the final model was used to develop a concept for a more individualized dosing scheme (section 3.5.3).

The final pharmacokinetic/pharmacodynamic model was a two-compartment model with first-order elimination. Clearance was estimated at 34.0 L/min, intercompartmental flow at 2.52 L/min, central volume of distribution at 336 L and peripheral volume of distribution at 85.6 L. Between subject variability was included in clearance and central volume of distribution, estimated at 30.8 and 34.4%, respectively. A common residual error was estimated for both studies at 34.8%. An effect compartment model with a sigmoid E_{max} response (Equation 7) was used to describe the relationship between DMT concentrations and subjective psychedelic intensity ratings. The equilibration rate constant was estimated at 1.74 min^{-1}, $EC_{50,e}$ at 102 nM and the Hill factor γ at 2.7. Between subject variability was estimated for $EC_{50,e}$ and γ at 69.1 and 64.7%, respectively. Estimates were similar for the intermediate model developed after the first part of the study. Overall, the estimates also concur with the previously published values in Paper II, further confirming, for example, the extremely high plasma clearance of DMT.

Some subjects appeared to develop an acute tolerance to DMT with regards to the psychedelic effects, with decreasing intensity ratings despite steady or increasing DMT levels. Thus, attempts were made to characterize this tolerance using different structural models [134-136]. However, some subjects did not exhibit any tolerance development and the large variability between subjects made it difficult to estimate any such parameters with acceptable precision.

The second part of the study was informed by the intermediate model derived from data from the first part of the study. Based on this model, the dose for the second part of the study was set to a 20 mg intravenous bolus dose followed by an infusion rate of 2.27 mg/min, with the aim of keeping subjects at ratings between 8 and 9. Recommended dose combinations were calculated using the

typical parameter estimates and the final dose decision was based on these recommendations as well as previous clinical experience with DMT. This dose resulted in observed median ratings around 8 over the time of infusion, i.e., within the predefined target. However, variability between subjects was large with ratings ranging between 2-10 during the time of infusion.

The results of the current study, as well as other previous studies [19, 21, 122], clearly indicate that the large variability between individuals makes it impossible to achieve psychedelic intensity ratings within a narrow target range in a large proportion of the population with a single dose regimen. Consequently, a concept for more individualized dosing of DMT, based on observed psychedelic intensity ratings, was developed. This concept was based on simulations (as described in section 3.5.3), with the intent to gradually adjust infusion rates, within a single session, based on reported ratings. This approach would potentially increase the proportion of the population within the target range. Based on simulations, the recommended starting dose was 1.2 mg/min as it led to the highest predicted proportion of the population within a target of 8-9 without more than 20% of the population exceeding the target range. As steady-state is reached, the dose rate is then adjusted depending on the reported ratings in each subject. These doses are set based on the predicted optimal dose in the simulated subjects below and above target. This simulation step can be repeated and if subjects are still below or above target as a new steady-state is reached, dose rates are adjusted again based on the results of these simulations. With this approach, the predicted proportion of the population within target increased from 18% after the starting dose, to 80% after two dose adjustments and 90% after three dose adjustments. For a more detailed description of the suggested doses, the reader is referred to Paper VI.

There are some limitations to this work. First of all, no information is available on covariates that could potentially explain some of the variability between individuals. If/when such information becomes available, this should be incorporated in the suggested framework. Secondly, as the recommended doses were based on simulations of ratings at steady-state, one needs to know when steady-state has been reached to know when to adjust the dose. As this cannot be concluded based on ratings alone, an assumption has to be made regarding when steady-state has been reached. One suggestion is to assume that most participants will have reached at least a pseudo steady-state after 20-30 minutes and that this would be an appropriate time point for dose adjustment. However, the variability in pharmacokinetics between individuals likely means that some subjects will not have reached steady-state at this point. Finally, while between subject variability has been taken into account in these simulations, the results could also be affected by between occasion and residual variability, making it even more difficult to define optimal doses on an individual level.

Nevertheless, we believe that the proposed concept could be a way forward in attempts to design more individually tailored dosing for DMT, granted that the currently suggested doses could be updated as more information becomes available in the future.

4.7 GENERAL DISCUSSION

As the papers included in this thesis describe a gradual development of the understanding of DMT pharmacokinetics and pharmacodynamics, there are some more general discussion topics, covering information from more than one paper at a time. This section aims to address some of those topics.

4.7.1 UNDERSTANDING THE ESTIMATED PHARMACOKINETIC PARAMETERS

The obtained pharmacokinetic parameters of DMT warrant some attention as they are unusual in a few different ways. Firstly, the estimated clearance of 34.0 L/min is extremely high. Something that is likely a consequence of the high affinity for MAO-A. Not only is the estimated clearance higher than the average hepatic blood flow (1.5 L/min), as already mentioned. It is also higher than average cardiac output in a healthy individual (~5 L/min), meaning that DMT clearance is completely independent of blood flow. While MAO-A is not, to the best of our knowledge, present in blood itself, it is known be present in the lining of blood vessels [120, 121], potentially explaining this phenomenon. It should however be pointed out that the clearance estimated here is that in plasma and not in whole blood. If DMT binds substantially to blood cells, it is possible that blood clearance is significantly lower than the presented plasma clearance. However, unpublished data after incubations of DMT in whole blood indicate that this is not the case.

Secondly, the estimated peripheral volume of distribution is lower than the estimated central volume of distribution. This is most commonly observed for hydrophilic compounds, where the drug usually distributes quickly in more hydrophilic tissue such as muscle (corresponding to the central volume of distribution), whereas it distributes more slowly and to a lesser extent into more lipophilic tissue (corresponding to the peripheral volume of distribution). However, DMT, with a logP of 2.57, has to be considered a lipophilic compound. Consequently, we would expect to observe the opposite, with a larger peripheral volume corresponding to a larger distribution into adipose tissue. A potential explanation for the unexpected results may be the short half-life of DMT. At the dose levels investigated here, concentrations are close to the limit of quantification at approximately 20 minutes after the administration of an intravenous bolus dose. Consequently, it may be that the full

pharmacokinetic profile of DMT is not captured. This would make estimation of the true terminal half-life and peripheral volume of distribution impossible without a considerable increase in dose or a more sensitive bioanalytical method. Another option to investigate this further would be to administer an infusion over a longer period of time than what has been investigated here. If the true terminal half-life is longer than estimated with the available data, this would become evident in the time it takes to reach steady-state. Nevertheless, the model appears robust without any substantial change in the estimates obtained in Paper II as compared to the final estimates in Paper VI.

4.7.2 APPROPRIATENESS OF USING SUBJECTIVE PSYCHEDELIC INTENSITY AS TARGET

Two of the papers included in this thesis (Papers V and VI) describe the development of different infusion regimens, where the target was based on subjective psychedelic intensity ratings. These ratings are measured on a scale of 0-10, where 0 is no psychedelic experience and 10 is the most intense psychedelic experience imaginable.

There are a number of limitations to using a subjective score to guide DMT dosing. The most obvious one being that the subjectiveness of the endpoint will likely lead to a large degree of variability between individuals, as different people might interpret the meaning of the scores differently. We would also expect a fair degree of inconsistency in the reporting of ratings for the same individual. Nevertheless, while we do observe a significant degree of variability both between and within individuals, it is clear that a fairly robust relationship between DMT exposure and psychedelic intensity ratings appears to exist. On the other hand, all of the subjects included in the studies reported here have previous experience with psychedelics. This has a potential impact on the reporting of scores and, consequently, the estimated DMT exposure-response relationship, as subjects with previous psychedelic experience may have a better sense of what would be expected to constitute an intense psychedelic experience as compared to subjects with no previous psychedelic experience. Consequently, there is a risk that variability would increase further if this concept is applied to the general population.

With the potential problems stemming from using a subjective score as the target for guiding DMT dosing, one may wonder if it would not be more appropriate to use a more objective measurement, such as the EEG data reported here, to guide dosing. However, there are also a number of advantages to using the psychedelic intensity scores. First of all, it is a cheap, non-invasive and simple method of measuring the response to DMT. While EEG or other biomarkers may be more reliable, they require more resources. Secondly, the intensity ratings can be obtained in real-time without the need for any data

processing and can consequently be used to guide dosing in a single session, as described in Paper VI.

Finally, one may also question the purpose of designing dose protocols based on a psychedelic intensity target. In the papers presented, we have assumed a target of ratings around 7-9, corresponding to a significant psychedelic experience while wanting to avoid ratings of 10, as too high doses may constitute a higher risk of adverse psychological reactions. There are a number of reasons for targeting an intense psychedelic experience. In the clinical studies presented here, the overall aim was to study changes in brain signaling during a psychedelic experience. Thus, it is an essential aspect of the study design to ensure that as many subjects as possible actually have a significant psychedelic experience. In addition, one could argue that this may be relevant also in studies aiming for therapeutic effects, in for example depression. While still unclear whether the psychedelic experience is necessary for therapeutic effects, correlations between the two have been observed [5, 6]. Consequently, the dose recommendations provided in this thesis may also be relevant in a therapeutic setting. However, more research is needed to draw firm conclusions regarding this.

4.7.3 GENERAL STUDY LIMITATIONS

The main limitation of the work presented within this thesis is the small study sample and the fact that no covariate analysis could be performed. The final pharmacokinetic model was based on a total of 36 individuals, which would technically allow for a covariate analysis. However, no covariate information was available. As the variability in the estimated parameters was large, this should be investigated further in the future.

Despite the small study sample, it should be pointed out that the amount of data is still fairly large. Dense pharmacokinetic sampling was performed with a total number of 621 DMT plasma concentrations to develop the final pharmacokinetic model. Unusually enough, the sampling of pharmacodynamic response was even more dense, with a total of 1608 psychedelic intensity ratings obtained in the two clinical studies. Hence, the final model presented in Paper VI should provide an adequate representation of DMT pharmacokinetics and pharmacodynamics.

Another limitation is the limited knowledge on DMT metabolism and the possibility that unidentified metabolites of DMT have not been accounted for. The pharmacokinetic model assumes that DMT elimination is via a single metabolic pathway, leading to the formation of IAA. Moreover, the final models assume that the pharmacodynamic response is elicited by DMT alone, i.e., without any contribution from active metabolites. As previously mentioned, we believe this to be an accurate representation of DMT

pharmacokinetics when administered intravenously as monotherapy. However, in Paper IV, DMT was demonstrated to be a substrate for CYP2D6, leading to the formation of oxygenated metabolites. It is possible that these metabolites may have pharmacological activity. More research is needed to confirm this and to investigate whether these metabolites are also formed *in vivo*. If this is the case, a model assuming a single elimination pathway of DMT may not adequately describe the full pharmacokinetics and pharmacodynamics of DMT after oral administration and/or in the presence of, for example, MAO inhibitors.

4.7.4 FINAL REMARKS

This thesis presents new knowledge on the pharmacokinetics and pharmacodynamics of DMT, including the first published population analyses of DMT data. It is also demonstrated how these models can be used to guide DMT dosing using simulations. This is demonstrated in practice in Paper VI, where an intermediate version of one of the pharmacokinetic/pharmacodynamic models was used to successfully set a dose for the second segment of the clinical study. It is also demonstrated in theory, in both Papers V and VI, how modeling and simulation could be used to further optimize an intravenous infusion protocol for DMT.

There are two main things that this thesis hopes to achieve. Firstly, the hope is that the information on DMT pharmacokinetics and pharmacodynamics presented here will be useful to anyone working with the clinical development of DMT. Secondly, as the interest in research with psychedelics in general is growing, we hope that this work can demonstrate the importance of understanding pharmacokinetic/pharmacodynamic concepts and the power of using a modeling and simulation approach within this context. The pharmacokinetic/pharmacodynamic knowledge is currently limited, not only for DMT, but for several other common psychedelic compounds. While the field of psychedelic research is moving forward, much more can be done with regards to optimizing doses and clinical trial designs based on a solid pharmacokinetic/pharmacodynamic understanding. All of the concepts presented here can be applied to any compound within this field of research and could potentially contribute to a more efficient development of psychedelics as therapeutic options.

5 CONCLUSIONS

This thesis investigated the pharmacokinetics and pharmacodynamics of DMT using preclinical and clinical data. The information obtained was used to design new dosing regimens for DMT. The main conclusions in this thesis are the following:

- A LC-MS/MS method was developed and validated for the quantification of DMT and two of its metabolites.
- The pharmacokinetics of DMT after intravenous administration was characterized using nonlinear mixed effects modeling. DMT concentrations were best described by a two-compartment model and the final estimate of DMT clearance was 34 L/min. This is a high value indicating that DMT clearance is independent of blood flow.
- The relationship between DMT and its psychedelic effects as well as its effects on the EEG spectrum were described using effect compartment models with inhibitory or stimulatory E_{max} responses. Out of the three investigated EEG measurements, alpha power appeared to exhibit the most robust exposure-response relationship and is suggested to be a more useful clinical biomarker compared to beta power and Lempel-Ziv complexity score. However, subjective psychedelic intensity was used to guide DMT dosing in this thesis as it is more easily obtained.
- DMT was shown to be a substrate for CYP2D6 *in vitro*, leading to the formation of oxygenated metabolites. Analysis with HR-MS indicates that this oxygenation likely occurs through hydroxylation on the indole core.
- The choice of using either a continuous variable model or a bounded integer model to describe the relationship between DMT concentrations and its subjective psychedelic effects had no major impact on decision-making around doses in the context of this thesis. However, the impact of model choice will depend on the predefined target.
- The applicability of the presented models was demonstrated through the design of optimized intravenous infusion regimens for DMT.
- A large variability was observed between individuals in both the pharmacokinetics and pharmacodynamics of DMT. Consequently, it is unlikely that a single dose can be used to achieve a target concentration or effect in a large proportion of a population. A concept for more individualized dosing, based on a modeling and simulation approach and reported psychedelic intensity ratings, was presented.

6 FUTURE PERSPECTIVES

While this thesis is an early step to better understand the pharmacokinetics and pharmacodynamics of DMT, much remains before DMT can be effectively and safely administered to a larger and more diverse population.

One of the main takeaways from the work presented here is that there is a large variability in both the pharmacokinetics and pharmacodynamics of DMT. This variability needs to be better understood to ensure optimal dosing of DMT in the future. Consequently, potential covariates that could affect the obtained exposure and response should be investigated in the future. This may include variables such as body weight, genetic polymorphisms in metabolizing enzymes and baseline neuropsychological factors. If such variables are identified, dosing could potentially be adjusted on an individual level based on individual characteristics.

Furthermore, the potential risk for drug-drug interactions also needs to be further investigated. This includes a deeper understanding of DMT metabolism as well as an investigation of DMT's potential to inhibit or induce metabolic enzymes. In this thesis, it was concluded that DMT is a substrate for CYP2D6 *in vitro*. Coadministration of DMT with CYP2D6 inhibitors/inducers could be studied to investigate the clinical impact on DMT exposure. This is especially important when DMT is administered in combination with MAO inhibitors, as is the case with ayahuasca, as this appears to shift the metabolic pattern of DMT, potentially making this pathway more important to the overall elimination of DMT.

Finally, this thesis aimed to demonstrate how pharmacokinetic/pharmacodynamic modeling and simulation can be used to guide dose decisions for DMT and potentially make the drug development process more efficient. However, for the models to be truly useful, therapeutic targets need to be established. In this thesis, dosing is based on target psychedelic intensity ratings. While we believe this to be a relevant endpoint, the target as such is relatively arbitrary. Studies are needed to establish what exposure level or psychedelic intensity is needed to achieve therapeutic effects as well as at what levels adverse events may occur. Before such a therapeutic window has been established, there is still uncertainty in the decision-making around appropriate doses of DMT in a clinical setting.

Overall, while some challenges remain, DMT research is moving forward quickly and there is great potential to solve the issues presented here with academic research groups as well as pharmaceutical companies getting involved in the field. Whether DMT is a viable therapeutic option or not remains to be seen, and it is clear that to be able to answer this question with

certainty, more research into the pharmacokinetics and pharmacodynamics of DMT is key.

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