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Quantification of Imipramine, Amitriptyline and Their Major Metabolites in Urine Samples of Depressed Patients by Gas Chromatography-Mass Spectrometry

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Abstract Biotransformation of antidepressant drugs is said to affect their pharmacological profile and subsequently alter their treatment efficacies. Imipramine and amitriptyline are the major drugs used for the management of manic depression at the Ankaful psychiatric hospital in Ghana. Recent reports have shown that some patients stay longer on this therapy than expected, while others do not get well at all. Herein, we report a modified solid phase extraction (SPE) method for the extraction of these antidepressant drugs; imipramine, amitriptyline and their respective metabolites, desipramine and 10-hydroxyamitriptyline from urine samples of five adult manic depressed patients. The compounds were simultaneously analyzed by gas chromatography-mass spectrometry (GC-MS). The calibration curves were linear over a range of 10-100 µg/L for all the analytes with the square of the regression coefficient (R2) ranging from 0.990 to 0.997. Analyses of the drugs and their respective metabolites over a 40 day period gave an average of 3.65 µg/L 10-hydroxyamitriptyline with negligible levels of amitriptyline. An average of 27.01 µg/L desipramine was also recorded against 10.30 µg/L of imipramine. These high levels of metabolites and relatively low amounts of the parent drugs recorded in the urine samples could be the main cause of the patients staying longer on the therapy and the resultant abscondment of one of them.

Key words: Gas chromatography-mass spectrometry, Solid phase extraction, Manic depression, Tricyclic antidepressant.

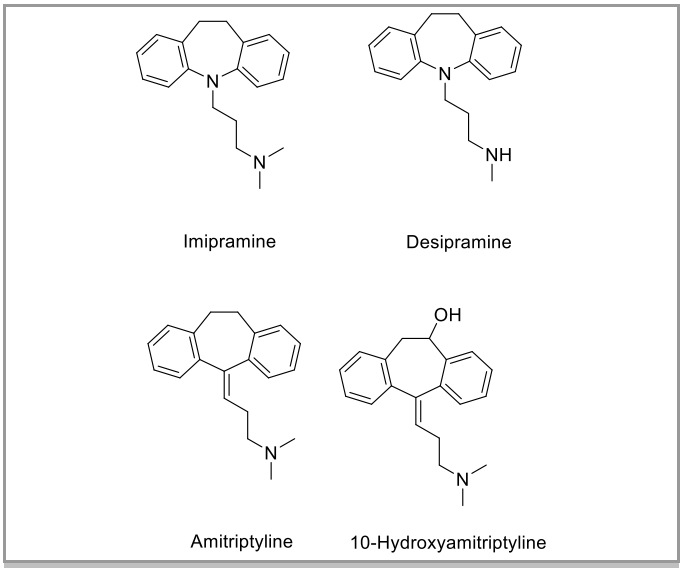

Figure 1. Chemical structures of the analyzed antidepressants and their respective metabolites.

1. Introduction

Amitriptyline (AT) and imipramine (IM) are important tricyclic compounds widely used as antidepressant drugs [1,2] with associated neuropathic pain reduction activity [3]. AT is marked under the trade names Elavil®, Laroxyl®, Seroten® and Tryptizol®; the corresponding IM is also available as Tofranil®. According to Coluzzi and Mattia [4] tricyclic antidepressants(TCAs) have been well known as noradrenaline and serotonin (5-HT) reuptake inhibitors which increase their bioavailability in the intersynaptic space. These neurotransmitters are important mediators in the management of depression and neuropathic pain. Similar studies by Reynolds and Miller [5] have shown that antidepressants have an N-methyl-D-aspartate (NMDA)-antagonistic like effect where they reduced NMDA –induced intercellular Ca^{2+} levels which have analgesic properties.

These drugs are usually administered orally or intramuscularly [6]. Breyer-Pfaff has shown clearly in a review [7] that AT undergoes N-demethylation to give the secondary amine, nortriptyline (NT), a process which is catalyzed by CYP enzymes in the human liver. The demethylation process does not result in the inactivation of AT but the produced NT has a different pharmacological profile. According to Nierenberg et al [8], nortriptyline has been successfully used for the treatment of treatment-resistant depression. AT therefore has pro-drug characteristics. NT has also been employed for smoke cessation with a magnitude of effect similar to that of bupropion and nicotine replacement therapies [9]. Other metabolites including amitriptylinoxide (AT-NO) and the N-glucuronide derivative have been reported in the urine of patients [10].

Hydroxylation at the ethylene bridge of the central ring also gives rise to enantio- and stereoselective isomeric alcohol metabolites. The E- and Z-10-hydroxyamitriptyline enantiomeric pair have being successfully determined in urine samples of depressant patients being treated with amitriptyline [11]. Metabolites of imipramine such as desipramine, 2-OH-imipramine, 2-OH-desimipramine, imipramine-N-oxide and didesipramine have been reported in blood samples of depressed patients [12] in urine samples [13,10] of patients with chronic forms of major depressions [14]. Although these drugs are administered to cure depressed patients, they end up producing metabolites with different pharmacological profiles.

Different methods such as gas chromatography (GC-MS) [10], nonaqueous capillary electrophoresis [13], liquid chromatography-mass spectrometry (LC-MS), liquid chromatography-tandem spectroscopy [15] and liquid chromatography/time-of-flight mass spectrometry have been reported for the analysis of metabolites of amitriptyline and imipramine. These methods have been proven to be fast, reliable and reproducible for the analysis of TCA metabolites in urine samples of patients. In this work, we report a simple method for the extraction of AT and IM and their respective metabolites, 10-hydroxyamitriptyline and desipramine, in the urine samples of five manic depressed patients on AT and IM psychotherapy at the Ankaful Psychiatric Hospital in the central region of Ghana. These are the main antidepressants used at the hospital facility. Generally, these drugs are supposed to offer some relief to the patients over a relatively short period of time based on the level of depression and the type of therapy given. It has been observed that some patients stay longer on the therapy than

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expected, some get worse and others even abscond. We therefore hypothesize that the parent drug is largely biotransformed into other metabolites thereby affecting the efficacy of the treatment. These samples were analysed using the GC-MS.

2. Material and methods

2.1. Standards and Reagents

Certified standard, amitriptyline (AM), imipramine (IM), desipramine (DES) and 10-hydroxyamitriptyline (10-HA) were obtained from Ernest Chemists Ghana Limited. Sodium hydroxide, hydrochloric acid, methanol and HPLC grade acetonitrile were purchased from Merck (Darmstadt, Germany). Potassium dihydrogen phosphate was also obtained from J. T. Baker (Deventer, Holland) with hexamethyldisilazane purchased from BDH Chemicals (London). Deionized water was used for all extractions.

2.1. Sample collection and pretreatment

Early morning urine samples were collected repeatedly from five (5) adult (40-45 years) patients being treated for manic-depression at the Ankaful Psychiatric Hospital, in the central region of Ghana. Each patient was given either 50 mgday-1 amitriptyline or 25 mgday-1 imipramine. Samples were taken twice weekly between 6:30 am and 10:00 am into Teflon containers. The urine samples were taken over a period of eight (8) weeks. The collected samples were preserved in an ice bath at a temperature below 4.0 oC and transported to the laboratory further treatment. At the laboratory, the samples were each acidified with two drops of hydrochloric acid in order to preserve the integrity of analytes [16].

2.2. Sample Extraction procedure

The extraction was performed in accordance with the standard protocol for analysis of drugs in biological samples[17,18] with minor modifications as follows. Exactly 200 µL of 0.2 M phosphate buffer was added to 2 mL of each urine sample, capped and swirled for 30 seconds to solubilize settled particles. Exactly 5000 µL of acetonitrile was added to each test tube and the mixture shaken for about 5 min following centrifugation at 3000 rpm for 10.0 min. The pH of the samples was then adjusted to fall between 6.0 and 7.0 with 2000 µL of 0.1 M phosphate buffer (pH 7.3). The bio-analytical C-18 solid phase extraction (SPE) cartridge was conditioned with 1000 µL of deionized water and 500 µL of acetonitrile. The sample was loaded unto the column and the cartridge was further washed with 1000 µL of deionized water followed by 1000 µL of a water/acetonitrile (1:1) mixture. The cartridge was left to drain slowly for 1.0 minute by applying positive pressure at the top using a syringe plunger. The analytes were eluted thrice and the total volume of eluent collected into clean dried test tubes.

The eluent was then concentrated under a stream of nitrogen and the concentrate reconstituted with 150 µL of acetonitrile to which a 100 µL of hexamethyldisilazane (HMDS, purity ≥ 99%) was also added as derivatizing reagent. The tubes were briefly mixed and incubated at 60 oC for 30 minutes using a water bath. Prior to GC-MS analysis of the derivatized extracts, the samples were reconstituted to 1.0 mL with acetonitrile.

2.3. Gas chromatographic-mass spectrometric analysis of samples

A mixed standard solution ranging from 10 to 100 µg/L amitriptyline, imipramine and their respective metabolites were prepared and derivatized in acetonitrile. These were used to generate the calibration curve for the quantification of each parent drug and the respective metabolite. The Shimadzu GCMS-QP2020 was used for identification and quantification of the analytes.

2.4. Gas Chromatographic Parameters

The GC was operated in splitless mode. Injection port temperature was maintained at 280 oC with a sampling time of 1.0 min using a linear velocity flow mode with a total flow of 50.0 µL/min, a column flow of 1.52 µL/min and linear velocity of 45.4 cm/sec. The sample injection volume was 1.0 µL. The initial oven temperature was kept at 80 oC which was held for 2.0 min before ramping at a rate of 10 oC/min to a final temperature of 290 oC and held at that temperature for 7.0 min. A total run time of 30 min was used for the separation. Helium gas (purity > 99.995) was used as a carrier gas. Chromatographic separation was achieved on a 30 m×0.25 mm× 0.25 µm ID Restek column from Shimadzu technologies (USA).

2.5. Mass Spectrometry Operation Parameters

The mass spectrometer was operated in selected ion monitoring mode (SIM) using electron impact ionization (EI = 70 eV). The ion source temperature was set at 230 °C whereas the interface temperature was set at 280 oC. This was also coupled with scanning at start m/z of 45.0 u to end m/z of 500.0 u.

3. Results and discussion

3.1. Linearity and Sensitivity of the GC-MS

The calibration curves for the analyzed compounds in the urine samples were linear over the range of concentrations analyzed. These calibrations were carried out using least squares linear regression analysis [19]. As shown in Table 1, all the curve fittings showed good coefficient of correlation with R2 values ranging from 0.990 to 0.997. The limits of detection (LOD) determined by running reagent blanks and limit of quantitation (LOQ = 3.3LOD) also ranged from 2.0 to 3.0 µg/L and 6.0 to 10.0 µg/L respectively. These results show the validity and reliability of the extraction method as well as the instrument for the quantification of the antidepressant drugs and their metabolites.

Table 1. Table 1: Gas chromatography-mass spectrometry characteristics of the antidepressant standards.

Compound	Linear range	n	Equation	R^2	LOD (µg/L)	LOQ (µg/L)
Amitriptyline	10-100	5	y = 0.196x + 8.204	0.990	2.0	6.0
10-hydroxyamitriptyline	10-100	5	y = 0.078x – 0.024	0.995	2.4	7.9
Imipramine	10-100	5	y = 0.022x + 0.062	0.996	2.0	6.0
Desipramine	10-100	5	y = 0.020x - 0.917	0.997	3.0	10.0
Amitriptyline	10-100	5	y = 0.196x + 8.204	0.990	2.0	6.0

3.2. Distribution of antidepressants and metabolites in urine samples of patients

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Figure 2 shows the levels of imipramine and its main metabolite, desipramine extracted from the urine samples of two manic depressed patients on a 25 mgday-1 dose over a 40 day period. These samples were collected after 5 hours of administration of the drug. For the first patient (figure 2A) high levels of the metabolite were recorded in all the samples. From an initial concentration of 298.82 µg/L desipramine recorded on the first day, the concentration dropped to 248.77 µg/L on the fifth day before reducing to 2.1 µg/L on the 8th day. Similarly, no imipramine was detected in the initial sample collected. As reported by Chen et al [20], imipramine is usually biotransformed in the liver into different compounds of which desipramine is a major metabolite.

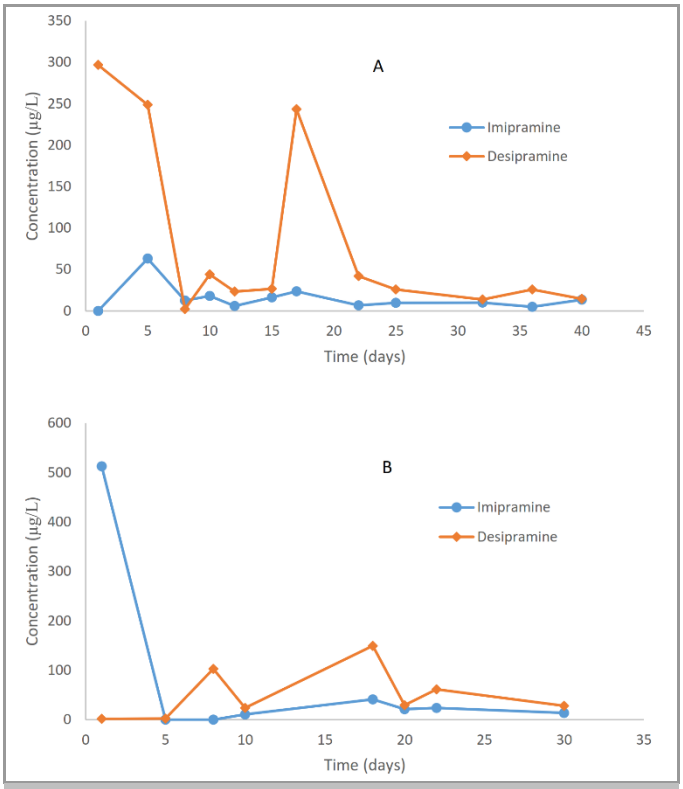

Figure 2. Levels of imipramine and desipramine in urine samples of patients on 25 mgday^{-1} dose over a 40 day period. A) patient 1 B) patient 2.

Generally, an average of 27.01 µg/L desipramine was analyzed in the urine samples collected over the 40 day period for patient 1 as compared to 10.30 µg/L recorded over the same period. This shows that the antidepressant, imipramine is usually demethylated into desipramine before performing its pharmacological function. Contrary to patient 1, patient 2 (figure 2B) recorded high levels of imipramine (513.03 µg/L) in the initial sample but this decreased below the detection limit for samples collected on the 5th and 8th days. The amount of desipramine however increased steadily from below detection limit to 103.04 µg/L over the same period. This confirms the observation made in figure 2A that imipramine is largely converted into active metabolites in the human body. As shown in figure 3, amitriptyline is generally biotransformed into 10-hydroxyamitriptyline. Earlier reports by Breyer-Pfaff have shown that hydroxylation of amitriptyline gives rise to a number of metabolites with 10-hydroxy compounds usually being the major products [7].

From figure 3A, 10-HA concentrations in the urine sample of the first patient was as high as 4.2 µg/L on the first day of analysis with negligible amounts of AM. The 10-HA concentration further reduced to 2.5 µg/L and then further dropped to 2.35 µg/L on the 10th day of analysis. On average, 3.82 µg/L 10-HA was recorded over the 35 days period of analyses with negligible amount of AM found in the urine sample. This shows that AM is largely biotransformed with negligible amount of the parent drug remaining unchanged. This patient however absconded before the end of the study. Analyses of the antidepressant and its metabolite in the urine sample of the second patient on AM treatment (figure 3B) showed similar trends as reported in the case of the 1st patient. High levels (55.26 µg/L) of the parent drug were reported from the beginning of the sampling. These levels reduced significantly below the detection limit for the subsequent days. The levels of 10-HA were however consistent with an average of 3.77 µg/L over the 40 days period.

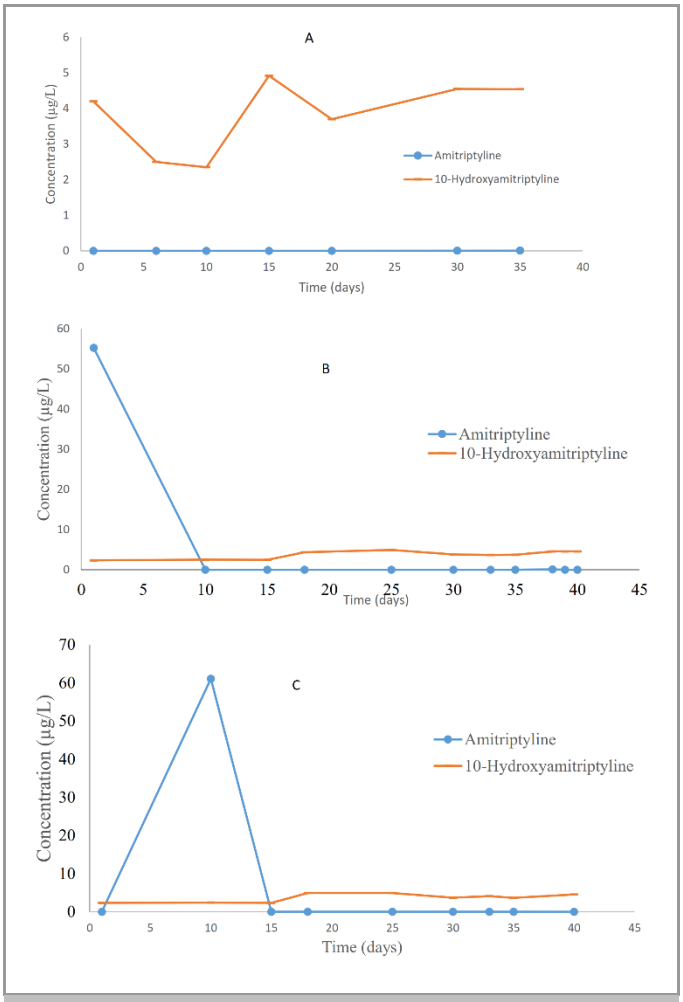

Figure 3. Levels of amitriptyline and 10-Hydoxyamitriptyline in urine samples of patients on 50 mgday^{-1} dose over a 40 day period. A) patient 1 B) patient 2 C) patient 3.

Similar trends were observed in the 3rd patient on the amitriptyline treatment (figure 3C) with an average of 3.65 µg/L 10-HA. All the analyses showed that AM is largely metabolized into 10-HA with negligible amounts of the parent drug present in the urine samples of the depressed samples analyzed.

Conclusion

A fast method for the extraction of imipramine, amitriptyline, desipramine and 10-hydroxyamitriptyline has been described and validated for the quantification of these compounds in the urine samples of patients with manic depression studied over a 40 day period. Gas chromatography-mass spectroscopy (GC-MS) was used for the analyses. The calibration curves were linear over a range of 10-100 µg/L with the square of the regression coefficient (R2) ranging from 0.990 to 0.997. Generally, imipramine was

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biotransformed into desipramine with an average concentration of 27.01 µg/L as compared to 10.30 µg/L of imipramine recorded over the same period. Similarly, amitriptyline was biotransformed into 10-hydroxyamitriptyline with an average concentration of 3.82 µg/L as compared to negligible concentrations of amitriptyline. There were clear indications that biotransformation of the antidepressant drugs affect the efficacy of the treatment leading to some patients not getting the desired effect.

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