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Analytical Studies on N-Benzylpiperazine and 1-(3-Trifluoromethylphenyl) piperazine- A New Class of Designer Drug of Abuse

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Abstract : The study focuses on analyzing two new emerging compounds that has gained popularity as an abused designer drug, Benzylpiperazine (BZP) and 1-(3-trifluoromethylphenyl)piperazine (TFMPP) through routinely practiced laboratory techniques include colour tests, GC-FID and GC-MS analysis. Marquis reagent test shows negative results for both compounds while Simon's reagent gives a blue complex for BZP and a slight faint blue complex with TFMPP. These two reagents in combination help to screen and distinguish the two piperazine analogues from amphetamine type compounds. A GC-FID method was developed to quantify BZP and TFMPP simultaneously since both often present as mixtures in seized pills. The GC method separated the two compounds. Linear curves in the range of 0.1 mg/mL – 1.0 mg/mL for both compounds were obtained with r^2>0.999. The precision of the method was 1.3% C.V for both compounds while method accuracy was determined to be -1.38% for BZP and -3.22% for TFMPP. GC-MS analysis gave qualitative results for underivatised BZP and TFMPP in their salt form with characteristic mass spectra. Street samples were analysed using these methods and confirmed the presence of both the compounds with caffeine being the adulterant. Erythrosine was also determined as the dye responsible for the colour of the tablets following a TLC assay. In conclusion, this simple, robust and rapid methodology of analyzing BZP and TFMPP was developed and validated for routine BZP and TFMPP analysis in narcotics laboratories.

Keywords : Beznylpiperazine, Trifluoromethylphenyl)Piperazine, Designer Drugs, Colour Test, Gas Chromatography, Forensic Chemistry

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Introductin

Designer Drugs had been gaining ground in the recent years within narcotics industry, in which they had been deployed as recreational drugs [1]. In the past decade, a group of drugs derived as piperazine related analogues, refers to as benzylpiperazines, have made a leap into the party scene with N-benzylpiperazine (BZP) together with its methylenedioxy analogue 1-(3,4-methylenedioxybenzyl)- piperazine (MDBP), and phenylpiperazines such as 1-(3-trifluoromethylphenyl)piperazine (TFMPP), 1-(3-chlorophenyl)piperazine (mCPP) and 1-(4-methoxyphenyl)piperazine (MeOPP) being the most commonly abused [2], **Figure 1**. Piperazines often found in the form of pills and can be mixed with adulterants such as caffeine [3, 4]. Loose powders are also occurring while solutions are rarely encountered [4] . They are available with the name of "party pills", "herbal highs" or "legal highs", and sometimes as tablets of ecstasy or amphetamine [5, 6]. Most prevalent piperazine blends are mixture of BZP and TFMPP, although blends up to four different piperazines (named X4) [6] or other drugs such as ecstasy, cocaine, ketamine and amphetamine [4] are possible.

The potential of piperazines as recreational drugs lies in their psychoactive properties, legal status and the perceived safety that ensued [7, 8]. This similar mode of action of the piperazines and the amphetamine type stimulants [6] gives the desired effects of euphoria, alertness and social activeness sought on the dance floor and thus gained its status as party pills [5, 9]. Potential adverse health effects [3] and potential of being abused lead to several countries, such as the United States, Australia, New Zealand, Japan and parts of Europe to have restricted the uncontrolled usage of these substances [10]. Malaysia at the mean time does not provide such a legal control [11]. Among the analogues, BZP is the most common derivative and is a labelled ingredient in a wide number of products on sale across the internet, under names such as "BZP", "A2", "Legal E", "Legal X", "Frenzy" and "Nemesis" [10, 12].

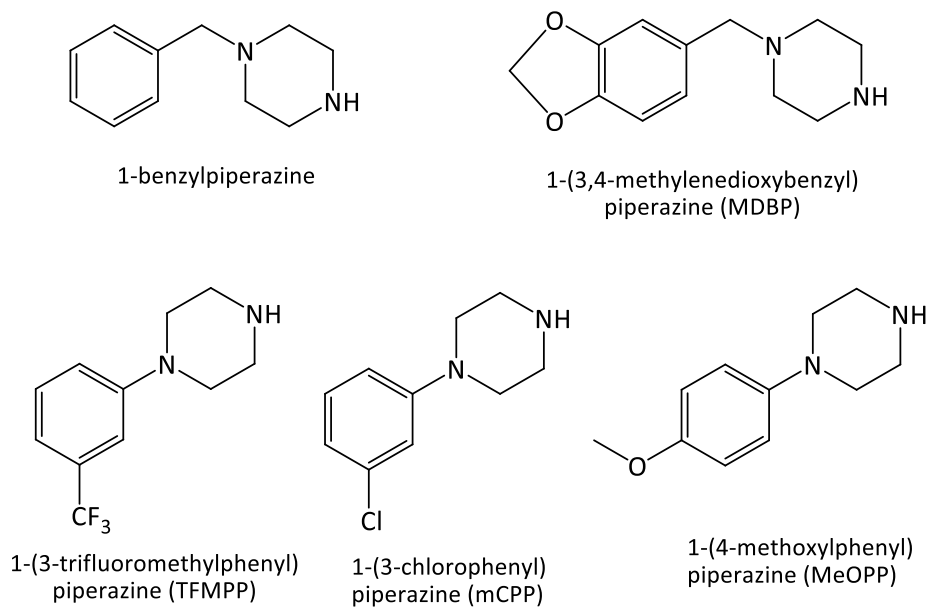

Figure 1 : General Abused Piperazine Analogues (Sourced and Adapted from Staack et al., 2007[6])

The dosage of BZP pills varies [5], capsule form of the drug under the name of A2 was found to have a dosage of 125 mg of BZP dihydrochloride, its common salt form [10]. Similarly, TFMPP, first encountered as a chemical intermediate in the synthesis of pharmaceutical product [2], is said to have behaved pharmacologically like other phenethylamines (MDMA, MDE) [3]. A combination of BZP and TFMPP is always advertised as a MDMA substitute. Both the drugs is reported to act in a synergy effect on each other [3], and as such, the pill with its stimulating, hallucinogenic and euphoric properties, as well as its easy availability has made its way into the club scene worldwide [12].

Recent seizure of a very large amount of pink "Adidas" pills containing BZP and TFMPP suggests the possibility that the drugs might have made its landing in Malaysian soil. In view of a potential prevalence of the drugs, we aim to develop a systematic scheme of work whereby seized sample could be screened, analyzed and quantitated using routine drug analysis chemicals and instruments, for the purpose of legal percussions, should such regulations exist in the near future. The study also seeks to verify the results produced by the compound through simple and daily practised laboratory tests, from colour screening tests, GC-MS qualitative analysis and subsequent GC-FID quantitative determinations. Chromatographic determination of underivatised BZP and TFMPP using common parameters of analysis in a narcotic laboratory is preferred and therefore investigated and discussed.

Experimental

Solvents, Chemicals, and Materials:

All solvents used were of analytical grade. Nortriptyline (≥98%, as hydrochloride salt) and TFMPP (99% purity, as monochloride salt) are obtained from Sigma Aldrich. BZP standard was obtained from Ambinter SARL (98%, as liquid base), caffeine standard (as base) and Silica gel 60 F_{254} were obtained from Merck. The BZP and TFMPP standards are then used to assay the purity of the lab's BZP.2HCl and TFMPP.HCl standards. Food colour standards, Amaranth, Erythrosine, Ponceau 4R, Red 2G, Rhodamine and Carmoisine were obtained from The Department of Chemistry Malaysia. Pink poster colour (Buncho®) was purchased from a local stationary shop.

Internal Standard Solution:

Internal standard solution was prepared by dissolving 0.60 g nortryptyline in a litre of methanol (0.6 mg / mL methanol).

BZP/TFMPP synthetic mixture:

A BZP/TFMPP tablet simulating synthetic mixture used for method validation is prepared by mixing 10.2060 g BZP-2HCl standards, 5.6493 g of TFMPP HCl and 10.4759g of caffeine manually in a clean Teflon bag, yielding a mixture BZP and TFMPP at 2:1 ratio as in commercial tablets.

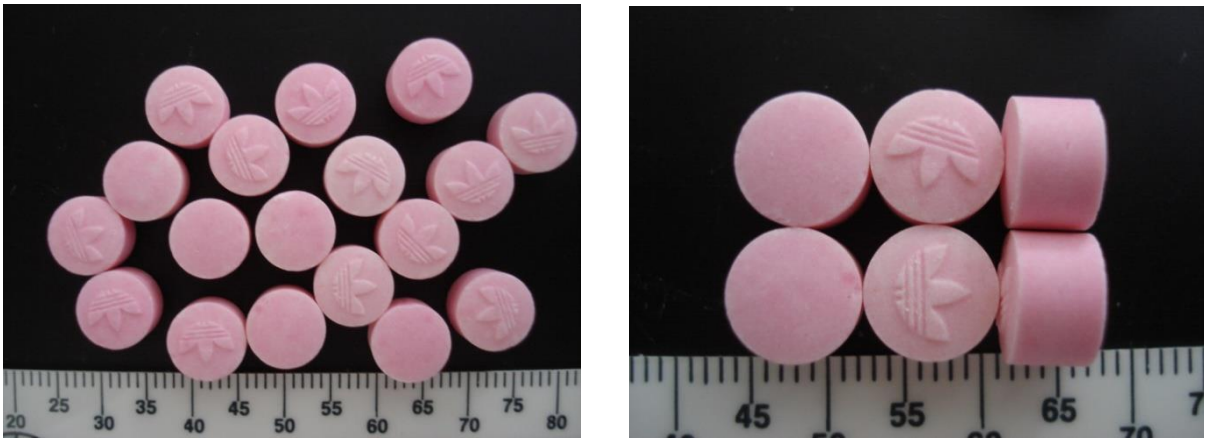

Figure 2 : General and Close up views of Different Orientations of the Pink "Adidas" Tablets.

Street Tablets:

Street samples of piperazine tablets were obtained from a clandestine laboratory seizure together with suspected powders of BZP and TFMPP, **Figure 2**. Two tablets (ca 360 mg/tablet with dimensions of 8mm x 8mm x 5mm) were pounded into fine powders with a pestle and mortar. 5.0 mg of the powder were dissolved in 1.5 mL of methanol (containing 0.6 mg nortryptyline/1 mL methanol) prior to GC analysis.

Colour Test:

The Marquis Reagent was prepared by mixing 2.0 mL of formaldehyde with 18.0 mL of concentrated sulphuric acid. The Simon's Reagent was prepared by dissolving 1.0 g of sodium nitroprusside in 50.0 mL of water. 2.0 mL acetaldehyde was then added to the solution through mixing. A small quantity of methamphetamine, amphetamine, ketamine, MDMA, BZP and TFMPP were placed on different areas of a spot plate. The reagent (Marquis Reagent or Simon's reagent) was subsequently added. Colour and physical changes were noted.

GC-MS Analysis

GC-MS analysis was performed with an Agilent GCMS 6890N equipped with a Agilent HP-5 (5% Phenyl Methyl Siloxane) capillary column (30 m length, 250 µm i.d. and 0.25 µm film thickness). The temperature of the injector and the interface was set at 250°C and 280°C respectively. The oven temperature was held at 150°C for 1 min and then raised to 220°C at a rate of 10°C/min followed by a rate of 90 °C/min to 290°C and held for 2.30 min. Helium was used as the carrier gas with an initial pressure of 110.4 kPa and at a flow rate of 1.2 mL/min. The 5975B MS detector operates in the ionization mode at ionization energy of 70eV. 5 µg of sample (i.e. BZP-2HCl, TFMPP-HCl, mixture of BZP-2HCl and TFMPP-HCl, and street sample in powder) was added into a GC vial and dissolved with 1.5 mL of methanol, respectively. The sample was voltex-mixed prior to GC-MS analysis.

GC-FID Quantification

The GC-FID analysis was performed by a Shimadzu 17A Gas Chromatography System equipped with a HP-5 (5% Phenyl Methyl Siloxane) capillary column (30 m length, 250 µm i.d. and 0.25 µm film thickness). The carrier gas used was helium at a flow rate of 1.2 mL/min. the injection and detector temperatures were kept at 270°C and 290°C respectively. The column temperature were initially programmed at 150°C for one minute and followed by a ramp of 10°C/min to 220°C, and finally with a rate of 90°C/min to 290°C with a hold time of 2.30 minutes. 1µL was injected and each sample was injected as two replicates.

To prepare the calibration curves, a combined stock solution of 5.0 mg/mL BZP dihydrochloride and TFMPP monohydrochloride was prepared by dissolving the calculated amount of BZP-2HCl and TFMPP-HCl in the internal standard solution (containing 0.6 mg nortriptyline/mL methanol). A series of standard solutions with BZP-2HCl and TFMPP-HCL (0.05- 1.0 mg/mL) were then prepared by pipetting the calculated amount of solutions directly from the stock solution and diluting with the same internal standard solution prior to GC-FID analysis.

The precision study was done by weighing accurately 10 samples of about 100 mg each of the synthetic mixture and dissolved in internal standard solution in 50 mL flasks. Each flask was then made up to 50 mL with the internal standard solution. The concentration readings were based on a single point calibration.

A standard addition assay was performed as follows: a solution of 5.0 mg/mL synthetic mixture, (see section 4.3) was prepared using internal standard solution (see section 4.2). 10.0 mL of this synthetic mixture solution was transferred into 6 flasks (25 mL). A mixed standard solution of 2.5 mg/mL of BZP and TFMPP in internal standard solution was then prepared separately. To these 6 flasks, none of BZP/TFMPP solution was added to the first flask, but in increments of 1.0 mL each in the subsequent five flasks. Each flask was then made up to 25 mL with the internal standard solution.

Thin Layer Chromatography:

To extract the dyes of the street samples, tablet powders were suspended in water in different plates before acidified with acetic acid. A piece of wool was then submerged and each plate was placed on a boiling water bath until the dye was transferred to the wool. The wool was then removed and washed thoroughly with warm water to remove extraneous materials. The dye on the wool was then re-extracted by warming the wool with 5 mL of a mixture of acetone and 3N ammonia solution (1:1) in a water bath for 5 min. The wool was then removed and the dye solution was carefully evaporated to dryness before reconstituted with methanol. The colouring agent in Buncho® poster colour in its gelatinous form was extracted with the same procedures. Dye standards were dissolved in methanol prior to TLC analysis using Silica gel and two solvent systems, i.e. (i) isopropanol:ammonia (4:1), and (ii) n-butanol:glacial acetic acid:water (10:5:6).

Results and Discussion

Colour tests:

The results of Marquis Reagent Test and Simon's Reagent tests on BZP and TFMPP, together with methamphetamine, ketamine and MDMA were tabulated in Table 1. The Marquis Reagent is known to work by the reaction of the carbonium ion from formaldehyde in the reagent with the aromatic structure of the compound. Under the influence of sulfuric acid, the carbenium ion is produced and stabilized by the coupling with a second molecule of the aromatic component, as shown in the case of amphetamines [13] by **Figure 3**.

Table 1: Reaction of Various Drugs with the Marquis Reagent and Simon's Reagent

Substance (Salt form)	Results and Observation	
	Marquis Reagent	**Simon's Reagent**
Blank	No physical change observed.	No physical change observed.
Methamphetamine	Brown orange complex formed	Dark blue complex formed
Ketamine	No physical change observed	No physical change observed
MDMA	Blue black complex formed	Dark blue complex formed
BZP	Slight fuming, but no colour change observed	Sea blue complex formed
TFMPP	No physical change observed.	Faint blue complex slowly formed

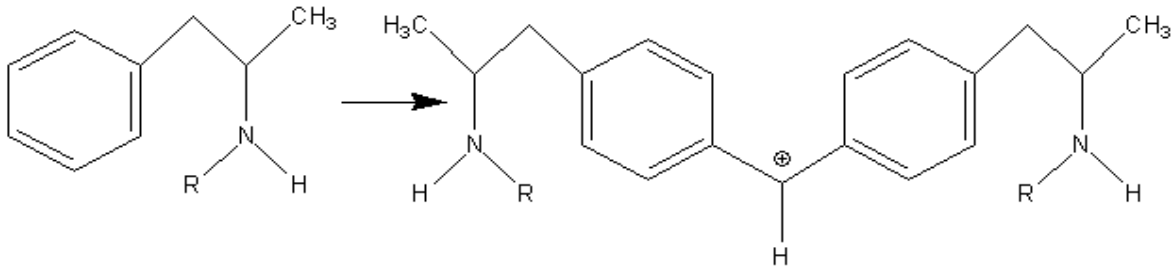

Figure 3: Reaction of Amphetamine (R=H) and Methamphetamines ($R=CH_3$) with the Marquis Reagent (Adapted from Kovar and Laudszun, 1989 [13])

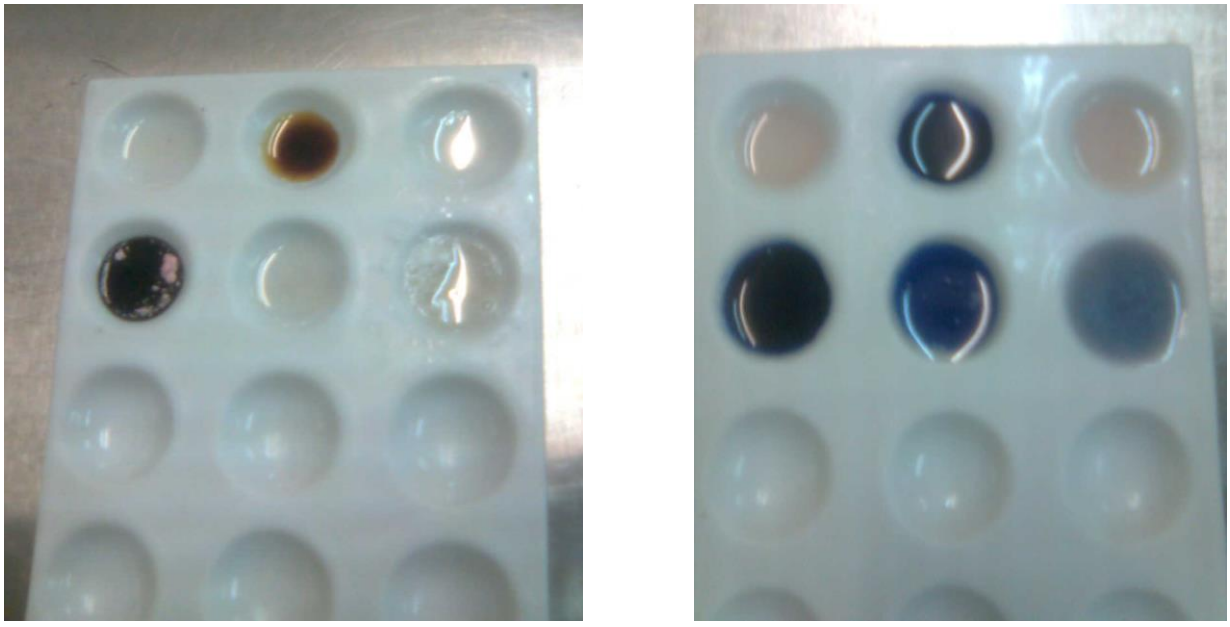

Figure 4 : Spot Plate on Test with Marquis Reagent (left) and Simon's Reagent (right). First Row (from left to right): Blank, Methamphetamine, Ketamine, Second Row (from left to right): MDMA, BZP and TFMPP.

The colour producing effect of the reagent is due to the formation of a relatively stable carbocation, in the form of a dimer of the original molecule with more conjugation that produce visible color appearances [14]. The reaction mechanism of the Marquis reagent itself is very complex and was not completely understood. Though the piperazines do contain the aromatic ring, they showed no colour reaction. Marquis Test alone is therefore not indicative of the piperazines as the negative reaction is also observed in other drugs such as ketamine. With Simon's reagent, BZP and TFMPP produce a colour reaction as they are also secondary amines such as methampheatamine and methylenedioxyamphetamine, Amine reacts with acetaldehyde from the reagent to produce an enamine, which subsequently reacts with sodium nitroprusside to an immonium salt that could be hydrolyzed to form the Simon-Awe Complex [13]. The production of blue colour in different shades suggested that standard samples of amphetamine type stimulants (ATS) or the piperazines should be used as positive control during testing of unknown samples so that the colours produced could be referred to for visual comparison. We suggest that both the Marquis Test and Simon's Test should be employed simultaneously in cases of BZP and TFMPP are suspected in seizures so that an indicative preliminary screening test could be undertaken. **Figure 4** shows changes brought about by the drugs on both the reagents.

GC-MS analysis:

The GC parameters employed separate both BZP (3.80 min) and TFMPP (3.99 min) in their salt forms, **Figure 5**. The mass spectrum of underivatised BZP had a base ion at m/z 91, with other ions at higher abundance being at m/z 134 and 176, **Figure 6**. The base ion for underivatised TFMPP was at m/z 188, with other ions at m/z 145, m/z 172 and m/z 230 being observed, **Figure 7**. GC-MS analysis of the salt form of these compounds without derivatisation is sufficient for the separation and identification under the experimental conditions. The GC-MS results do partially supports the work by Inoue *et al*. [12] in which the non-derivatized approach was sufficient to resolute both BZP and TFMPP, but our work also gives confidence to the fact that the salt form of the drug could be directly utilized for its identification in GC-MS without a conversion into the base form.

A small peak at the retention time of about 9 min was observed and identified as dibenzylpiperazine (DBZP). DBZP is a known impurity that came from the clandestine mechanism of synthesis for BZP diHCl [4]. An analysis of the seized sample yields only TFMPP as the major ingredient, together with caffeine (R.T = 6.86 min), a common adulterant for piperazine tablets [15].

The presence of sharp peaks and good separation for the two compounds of interest BZP and TFMPP is good, with the only setback is that peak tailing was quite evident. Nevertheless, the integration approaches by the software and also

the presence of the sharp peaks is capable of overriding this problem. As far as the qualitative aspect is concerned, such minor asymmetry at the base of the peak is not a big issue

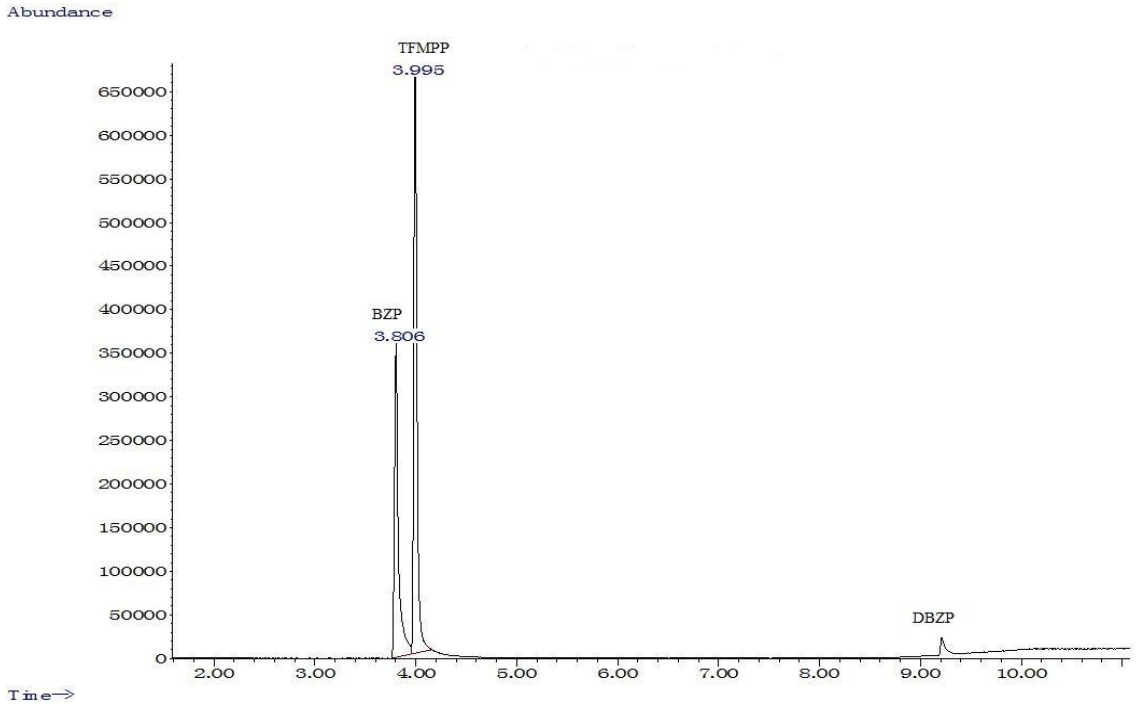

Figure 5 : Gas Chromatogram of the Standard Mixtures Showing BZP (RT=3.806 min) and TFMPP (RT= 3.995min)

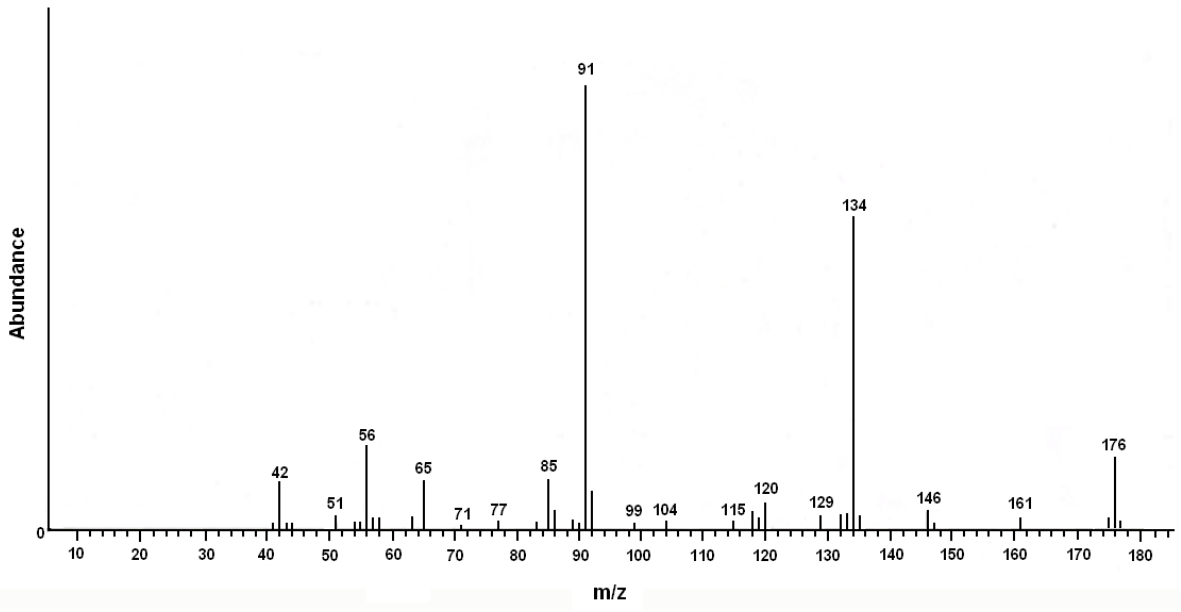

Figure 6 : GC-MS mass spectrum of BZP

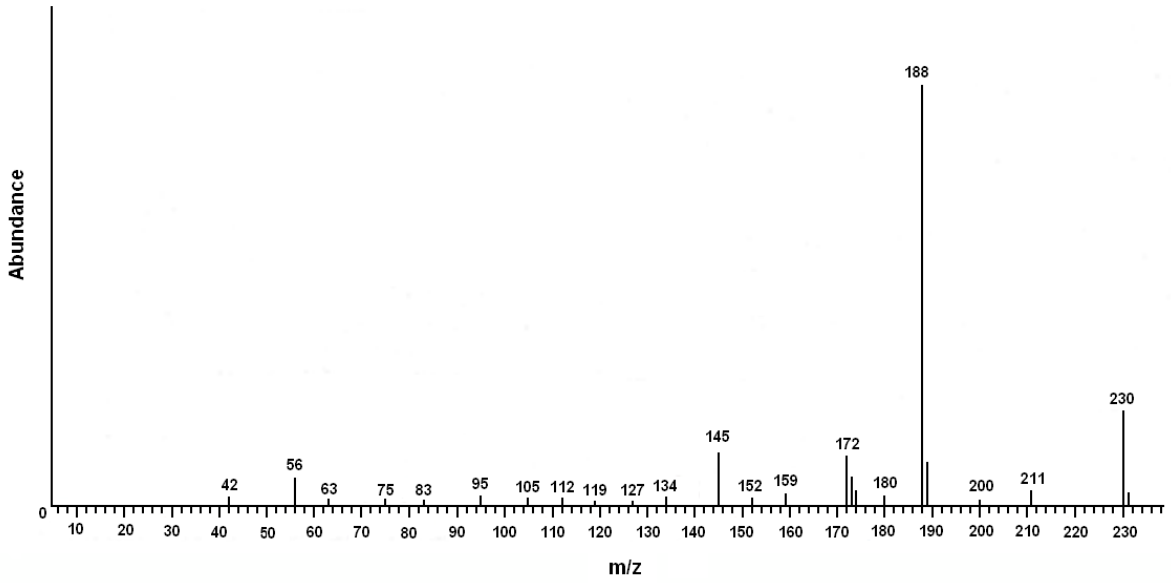

Figure 7 : GC-MS mass spectrum of TFMPP

GC-FID quantification:

he GC parameters used for the GC-FID produces a similar chromatographic separation to the chromatograms of the GC-MS, as shown **in Figure 8**. The method linearity for both BZP and TFMPP was determined over the concentration range stated in the Experimental section (0.05-1.0 mg/mL). The calculated correlation coefficient value (R^2) was over 0.999 from the calibration curves which were plotted using the peak area ratio between the compound and the internal standard against the concentration of the standards, **Figure 9** and **Figure 10**, respectively. The method precision was determined using 10 samples of similar concentration as listed in the Experimental section.

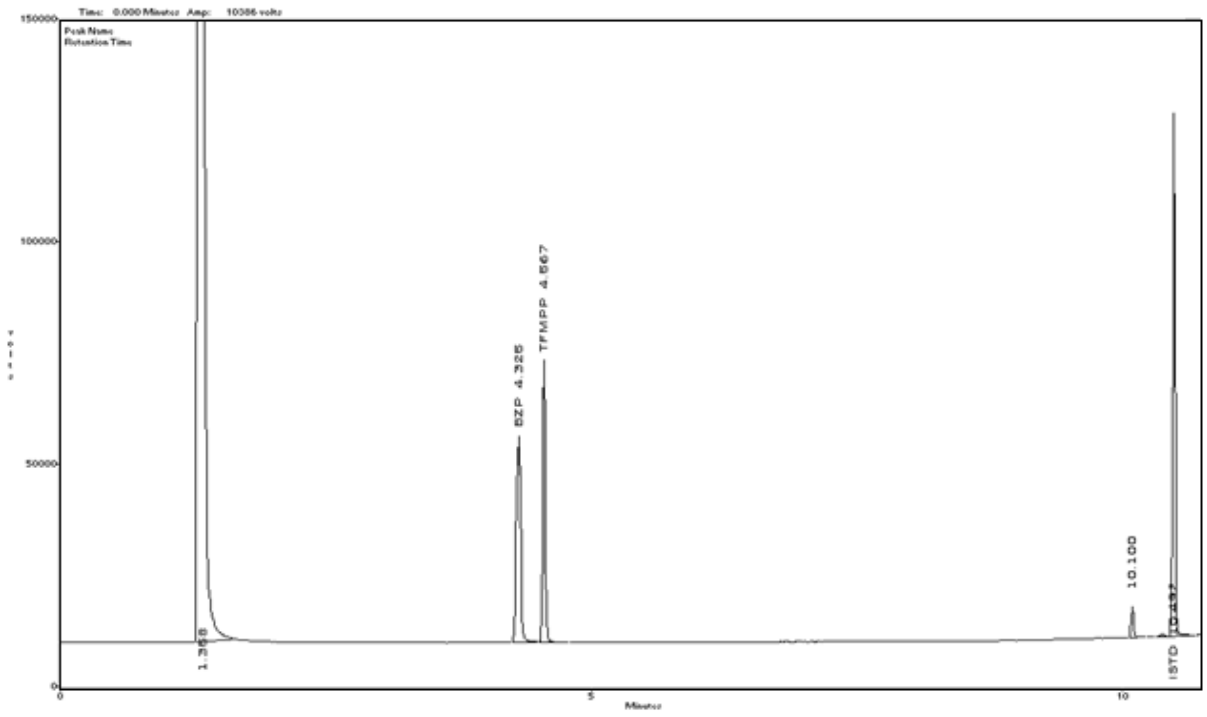

Figure 8: GC-FID generated separation for the Standard Mixture

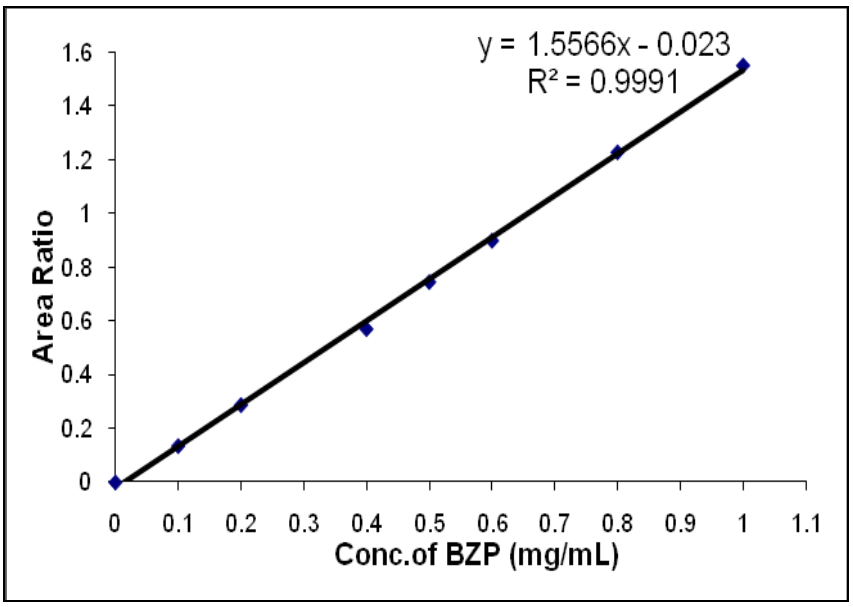

Figure 9 : Calibration Curve with Area Ratio between BZP and Internal Standard against Concentration of BZP

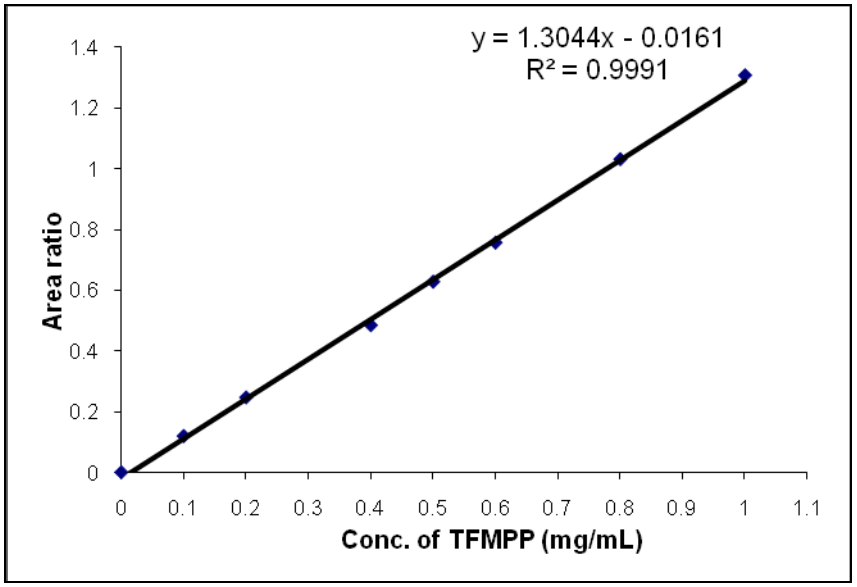

Figure 10 : Calibration Curve with Area Ratio between TFMPP and Internal Standard against Concentrations of TFMPP

The Relative Standard Deviations (RSDs) were 1.36 % for BZP and 1.39 % for TFMPP, with a recovery of 100.52% for BZP and 98.89% for TFMPP. The method accuracy was determined by standard addition method, yielding a recovery of 98.5% for BZP and 98.2% for TFMPP. The precision and accuracy values are clearly within the range of acceptability [16]. Slight tailing and resolution issues of the chromatograms should be easily offset by the significant values as shown in the accuracy and precision assay, not to mention that our samples are all in their salt form, all are bound to show some matrix interference.

Quantitation of the tablets in triplicate yielded an average of 26.0 % and 17.6 % BZP and TFMPP base respectively. The identity of the tablets as well as its composition of BZP and TFMPP matches closely with our synthetic mixture that was used for the method validation process. Note that the pink tablets were used without any means of filtering or extraction prior to GC analysis.

Table 2 : Retention Factors of the Various Colourants Using Two Solvent Systems

Standards/ Samples	**Retention Factor (R_f)**	
	Isopropanol/ammonia (4:1)	**n-butanol/glacial acetic acid/ water (10:5:6)**
Colourant from seized tablet	0.67	0.91
Buncho Pink	0.60	0.56
Carmoisine	0.33	0.35
Erythrosine	0.67	0.91
Ponceau 4R	0.02	0.07
Rhodamine	0.28	0.23
Red 2G	0.36	0.68
Amaranth	0.10	0.37, 0.67

TLC analysis:

The results of TLC analysis were tabulated in **Table 2**. Note that Buncho® poster colour was included as it was found at clandestine laboratory dismantled besides the similar hue of the tablets to the poster colour. TLC results suggested that the colour from the tablet comes from erythrosine. The second solvent system excluded the poster colour as the colouring agent. The wool extraction technique was found reliable in extracting the colouring substance from tablets as well as the poster colour consisting of a matrix of various components like pigment, glycerine, kaolin clay, dextrin aqueous solution, surfactant and others [17].

Conclusion

A framework of analysis of the BZP and TFMPP has been developed, from spot test screening to conclusive qualitative and quantitative analyses. Due to the simplicity of the methodology which requires no derivatization and base conversion, it could be easily adopted for forensic narcotics laboratories that normally receive BZP and TFMPP in their contraband form for simpler and quicker forensic analysis. Testing with more samples from the amphetamine and piperazine in the qualitative as well as the quantitative assay would certainly add credit and confidence to our presented methodology.

Acknowledgements

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