



Rapid analysis of the skin irritant *p*-phenylenediamine (PPD) in henna products using atmospheric solids analysis probe mass spectrometry



Weiyang Chen^a, Thobile. A.N. Nkosi^a, Sandra Combrinck^{a,b}, Alvaro. M. Viljoen^{a,b,*}, Catherine Cartwright-Jones^c

^a Department of Pharmaceutical Sciences, Faculty of Science, Tshwane University of Technology, Private Bag X680, Pretoria 0001, South Africa

^b SAMRC Herbal Drugs Research Unit, Faculty of Science, Tshwane University of Technology, Private Bag X680, Pretoria, 0001, South Africa

^c 339 Tallmadge Rd., Kent, OH 44240, USA

ARTICLE INFO

Article history:

Received 17 February 2016

Received in revised form 10 May 2016

Accepted 11 May 2016

Available online 12 May 2016

Keywords:

Lawsonia inermis

Henna

para-Phenylenediamine (PPD)

Atmospheric pressure solids analysis probe

ABSTRACT

Henna (*Lawsonia inermis*) is applied to stain keratin, present in hair, skin and fingernails, a red-orange or rust colour. Producers of temporary tattoos mix the aromatic amine compound, *para*-phenylenediamine (PPD) into natural henna to create 'black henna' that rapidly stains the skin black. However, PPD may cause severe delayed hypersensitivity reactions following skin contact. This study proposes a rapid direct-analysis method to detect and identify PPD using an atmospheric solids analysis probe (ASAP) coupled to a Q-ToF mass spectrometer (MS). Since laborious, multistep methods of analysis to determine PPD are undesirable, due to the instability of the compound in solution, a screening method involving no sample preparation steps was developed. Experiments were carried out to optimise the corona current, sample cone voltage, source temperature, and desolvation gas temperature to determine ideal ASAP-Q-ToF-MS analysing conditions. Eleven of the 109 henna samples, originating from various countries, tested positive for PPD when henna products were screened using ASAP-MS, without any form of sample preparation other than grinding. Ultra-performance liquid chromatography electrospray ionisation-mass spectrometry (UPLC-Q-ToF-MS) was subsequently used to confirm the results from ASAP and to determine the concentrations of PPD in henna products. The allergen was detected in the same eleven samples, with concentrations ranging from 0.05–4.21% (w/w). It can be concluded that the sensitivity of the ASAP-MS technique is sufficient (limit of detection = 0.025% w/w) to allow screening of henna samples for the presence of PPD. This relatively new technique can be applied to commercial products without extraction, sample treatment or chromatographic separation.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Lawsonia inermis, a shrub or small tree commonly known as henna, belongs to the Lythraceae family and is the only member of the genus *Lawsonia*. It is cultivated commercially in India, Egypt, Somalia, Sudan, Morocco, Iran, Yemen, Niger and Pakistan, with India being the largest producer [1]. Henna leaves produce an orange-red naphthoquinone dye, known as lawsone. Paste prepared from powdered henna is traditionally used for dyeing hair; henna extracts are also incorporated into other cosmetic products. There is evidence of henna being applied to mask grey hair in North East

Africa as early as 6000 BCE and to stain skin since the Bronze Age in the eastern Mediterranean. The plant material was, and still is, traditionally applied to the hands, nails and feet as durable, but not permanent, adornments for weddings and other festivals in cultures originating from regions where the plant flourishes. The cultural use of henna was transmitted beyond its native botanical zone through the expansion of Islam throughout Africa, the Levant, the Middle East, and South Asia, as far as Malaysia and Western Australia. Since 1998, henna and 'black henna' temporary tattoos have globalised into western popular culture.

Natural henna is regarded as safe for topical application with very few reports of allergic reactions, despite its extensive use [2]. Although the application of henna has been known to cause oxidative haemolysis in neonates and juveniles who suffer from homozygous glucose-6-phosphate dehydrogenase deficiency [3], allergic reactions to topical applications of henna products are extremely rare [4]. Virtually all reported cases of hypersensitivity

* Corresponding author at: Department of Pharmaceutical Sciences, Faculty of Science, Tshwane University of Technology, Private Bag X680, Pretoria 0001, South Africa.

E-mail address: viljoenam@tut.ac.za (Alvaro.M. Viljoen).

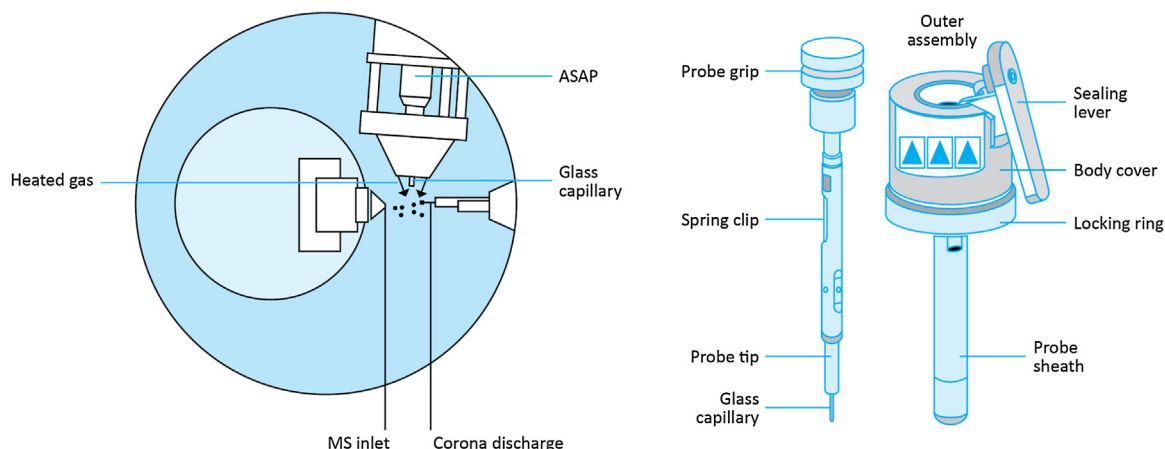


Fig. 1. Schematic of the ASAP source and probe assembly (Modified with permission from WATERS).

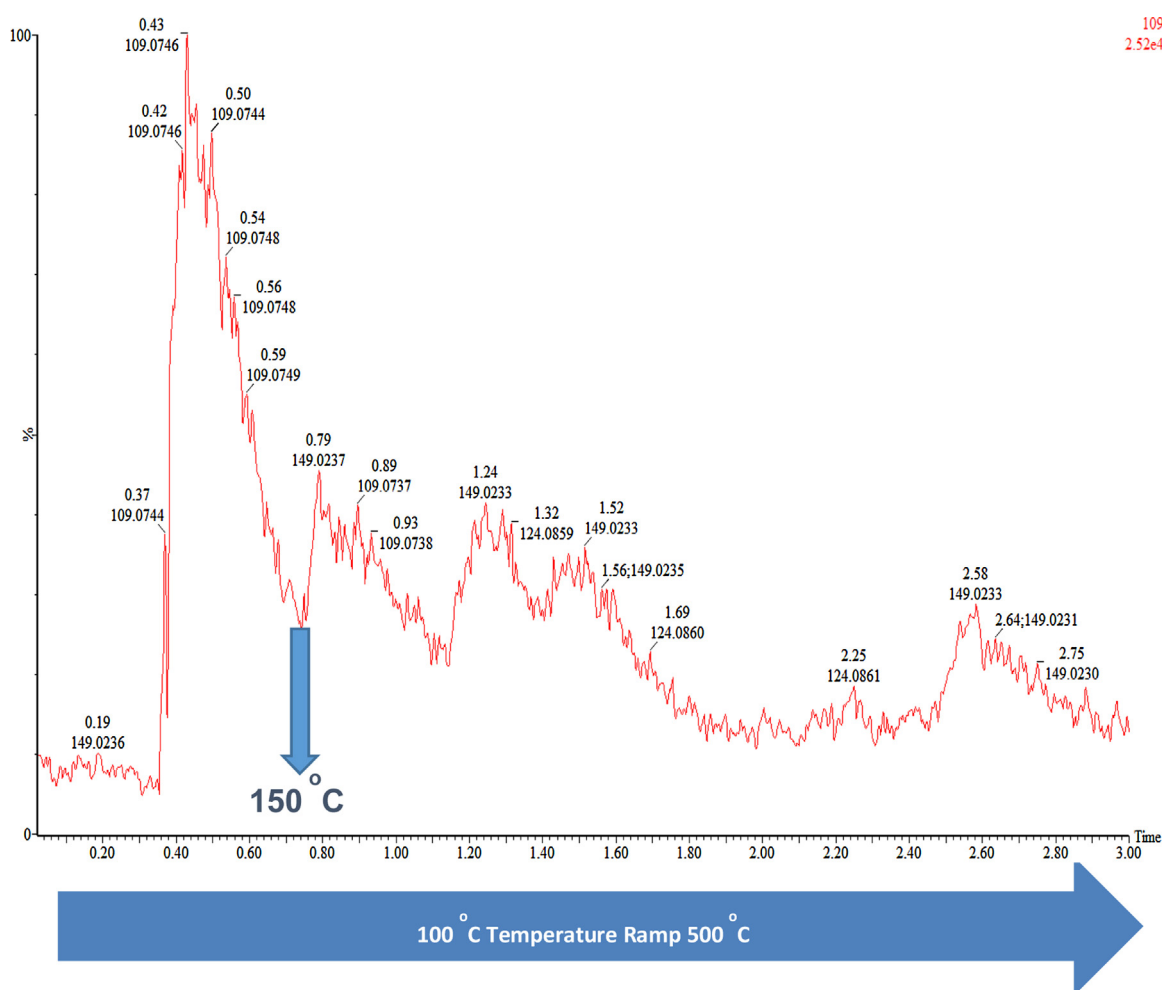


Fig. 2. Effect of ASAP ramp desolvation temperature on the extracted MS ion trace of PPD.

were associated with the adulteration of natural orange-red colour henna with *p*-phenylenediamine (PPD) [1].

People adapted 'black henna' into body art to create fast, black artistic temporary tattoos when PPD-based black hair dye became available as a home hair dye kit in east Africa in the 1970's [5]. Whereas henna paste must be in contact with keratin for four to eight hours to create a saturated stain, the addition of PPD results in a durable tattoo in less than an hour. This rapid and efficient

colouring action is due to the excellent protein-binding capacity of PPD and its rapid penetration into the hair shaft [6]. The compound is an aromatic amine with chemical formula $C_6H_8N_2$ and has a molecular weight of 108.15 g/mol. It is applied as an azo dye intermediate for dyeing fur, is used for photochemical measurements and as a photographic developing agent, and serves as an intermediate in the manufacture of antioxidants and accelerators for rubbers [7].

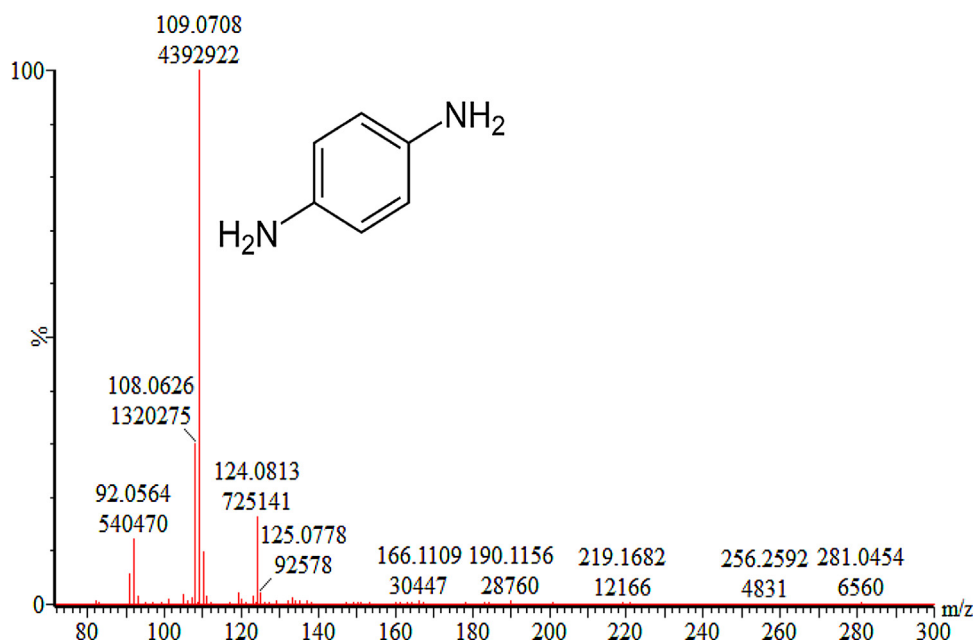


Fig. 3. ASAP-Q-ToF mass spectrum (positive ionisation mode) of PPD.

p-Phenylenediamine added to, or substituted for, henna, to create a fast, black skin stain, is considered more modern, more efficient, more attractive, and more nearly resembling black permanent tattooing. The patent holder, DuPont, "... does not recommend and will not knowingly offer or sell *p*-(PPD) for uses involving prolonged skin contact. Such uses may involve, but are not limited to, products formulated with henna for tattoo applications or other skin coloration effects. This use of PPD in prolonged skin contact applications has the potential to induce allergic skin reactions in sensitive individuals" (<http://www2.dupont.com/>). A patch augmented with 10% PPD was found to cause 100% sensitisation in individuals after one to five exposures [8]. Some of the adverse health effects associated with the use of 'black henna' containing PPD are contact dermatitis, palpebral eczema, blistering similar in appearance to chemical burns, life-long sensitisation and cross-sensitisation to diamines and chemically similar coal tar derivatives, acute renal failure, acute and severe angioneurotic edema, abdominal pain and vomiting [9,10]. Some researchers have expressed concern that the oxidation of PPD during hair dyeing may lead to the formation of a mutagenic compound known as Bandrowski's base [11].

Various techniques, including thin layer chromatography (TLC), high performance liquid chromatography (HPLC), liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) and gas chromatography-mass spectrometry (GC-MS), have been applied to determine the presence of PPD in hair dyes and henna powders [6,12,13]. Sample preparation and chromatographic separation are common bottlenecks in analyses, since these steps are often tedious, time-consuming and expensive. Mohamed et al. [13] reported that the concentration of PPD in a blood sample was diminished to only 3% of its original concentration after 24 h, during a stability evaluation. To mitigate the oxidation of PPD, they added ascorbic acid as antioxidant. However, PPD proved to be a stronger reducing agent than ascorbic acid, making it difficult to prevent the reaction when PPD is exposed to air and/or light. Samples must therefore be extracted within a short time and stored in amber vials prior to analysis to minimize the risk of oxidation. The use of a direct, rapid and inexpensive analytical technique, implemented to detect the presence of PPD, could be extremely valuable as part of a quality control regime for henna products.

Developed in 2005, the atmospheric solids analysis probe (ASAP) [14], can be applied directly to a broad range of diverse solid or liquid samples, without complex sample preparation and chromatographic separation, to rapidly determine the presence of volatile and semi-volatile compounds. All ionisable, volatile analytes in the sample reach the source and are detected by the mass spectrometer (MS), in a full scan experiment, thereby minimizing any analyte losses. The technique has been successfully applied to the analysis of drugs, natural products, nucleosides, polymers, coal-related model compounds and steroids [15–17].

A significant advantage of ASAP is that the sample can be introduced directly into the mass spectrometer at atmospheric pressure without the need for a source vacuum [17]. Since the technique is based on atmospheric pressure chemical ionisation (APCI), simple mass spectra that typically show to the molecular ion $[M+H]^+$ are produced in positive mode [18,19].

The basic principle of ASAP ionisation (Waters, USA) is illustrated in Fig. 1 [20]. A liquid or solid sample is deposited on the tip of the sealed glass capillary of the probe by simply touching the sample with the capillary. The probe is subsequently inserted directly into the mass spectrometer where a flow of heated gas causes thermal desorption of the compounds present. Ionisation of the analyte occurs through proton or charge transfer reactions. The proton source can be water, but methanol or other solvents can be used [21]. The production of protonated molecules relies heavily on the molecular interaction/charge-transfer process occurring on the sample surface. Since specific ion masses are extracted, the ASAP probe constitutes a powerful screening tool for the presence or absence of analytes. Several key parameters, including the corona current, sample cone voltage and gas desolvation temperature, require optimisation because these variables determine the total number of ions detected [22]. The ASAP can be used in conjugation with quadrupole ToF-MS (Q-ToF-MS) to produce high resolution and obtain accurate mass measurements.

The purpose of this study was to assess ASAP-Q-ToF-MS as a rapid screening technique to determine the presence of PPD in henna samples, without extraction or sample pre-treatment. The validity of the results and the sensitivity of the technique towards PPD were tested using UPLC-Q-ToF-MS as a quantitative method.

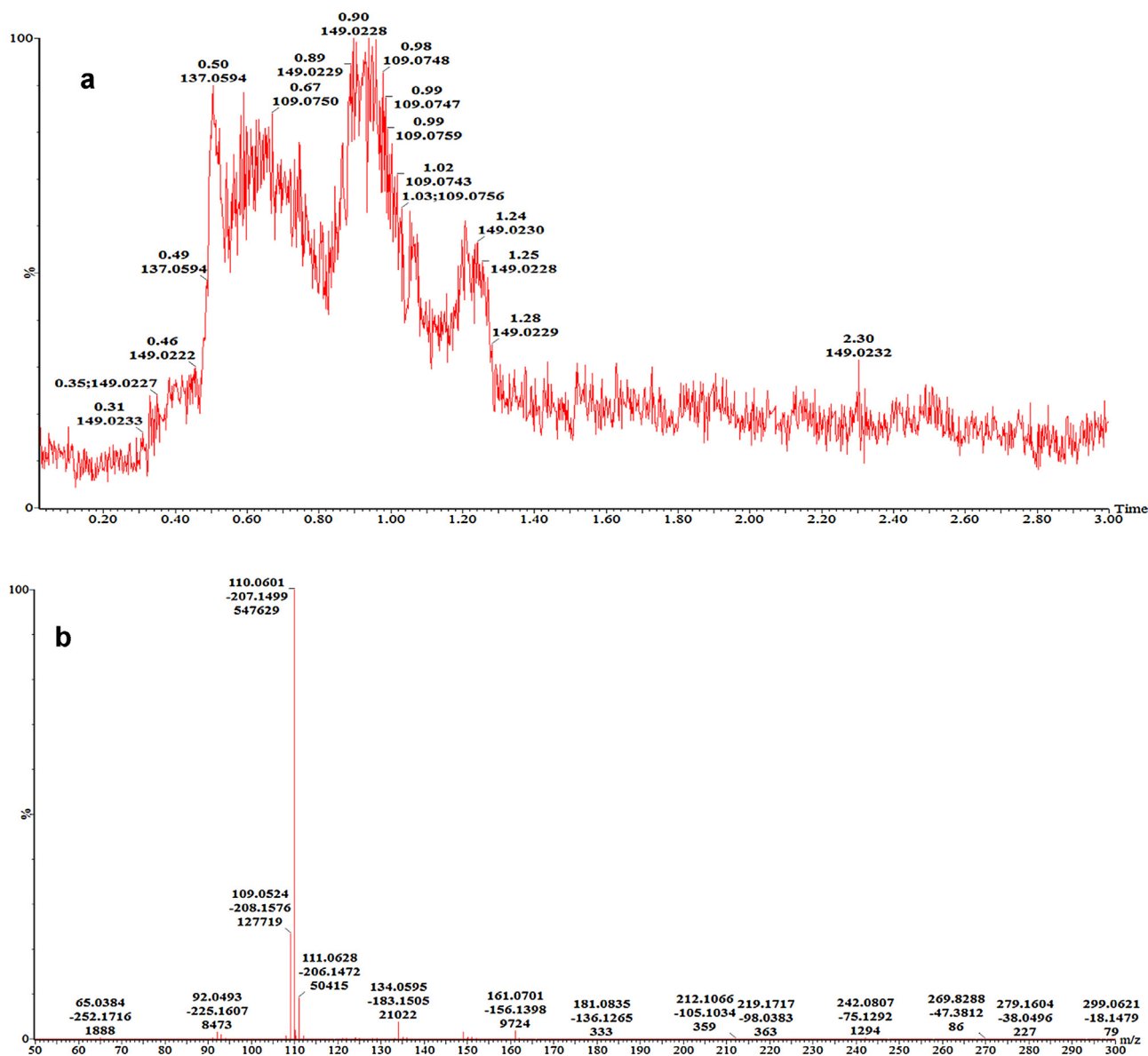


Fig. 4. (a) Extracted ion pseudo chromatogram of henna sample containing PPD by ASAP. (b) The mass spectrum corresponding to PPD.

2. Materials and methods

2.1. Chemicals and reagents

A reference standard of PPD (purity 100%) was purchased from CAPOT (Hongzhou, China). Acetonitrile and methanol were HPLC grade (Romil, Cambridge, UK), while ethanol and formic acid (SMM Instruments, Johannesburg, South Africa) were analytical reagent grade.

2.2. Sample preparation

Henna samples (109) from 16 countries were purchased from retail exporters selling packets of henna on Ebay in March 2013. These samples were powdered using a Retsch® 400 MM ball mill (Haan, Germany) and sieved through a 500 µm sieve (Endecotts Ltd., London, UK). For UPLC–Q-ToF-MS analysis, 50.00 mL of methanol (HPLC grade) was added to each accurately weighed sample (250 mg). The mixture was sonicated (Branson 2510, Mexico) for 15 min at room temperature and subsequently filtered

through a 0.2 µm nylon syringe filter (Bonna-Agela Technologies, Wilmington, DE, USA) prior to analysis.

2.3. ASAP-Q-ToF-MS analysis

The PPD was determined using an ASAP coupled to a XEVO G2 Q-ToF mass spectrometer (Waters Corp., Milford, MA, USA). Each henna sample was introduced directly into the ion source after dipping the end of the capillary, pre-wetted with methanol, into the sample powder. Heated nitrogen gas was applied to desorb the sample. Three parameters i.e. the corona current (µA), which affects the ionisation, sample cone voltage (V), which influences the in-source fragmentation and attracts charged ions into the mass analyser region, and the desolvation gas temperature (°C), which affects volatilisation, were optimised by repeatedly analysing the PPD standard in methanol (100 µg/mL). The standard was analysed by dipping the capillary into the solution, and removing the excess. The corona current was investigated over the range 3–6 µA, the sample cone voltage (V) was increased from 10 to 40 V, while the desolvation gas temperature was manually varied from 100 °C

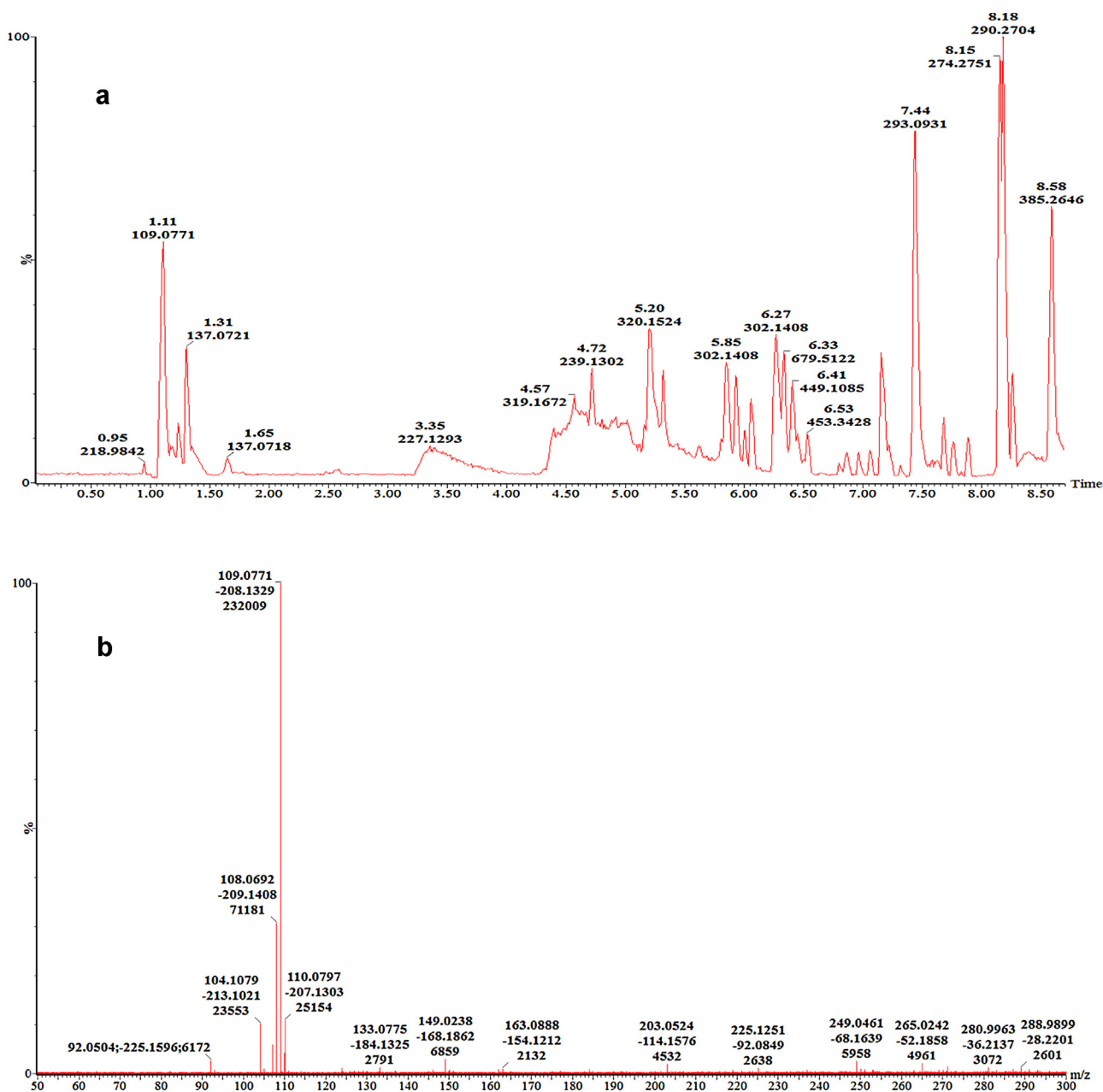


Fig. 5. (a) Base peak intensity (BPI) UPLC-Q-ToF-MS chromatogram of a henna sample containing PPD. (b) The mass spectrum corresponding to PPD.

to 500 °C in 50 °C steps over 20 s intervals. The main purpose of optimisation was to produce as many ions from PPD as possible, thereby reducing in-source fragmentation to facilitate further identification. To achieve this, one key parameter was changed in turn, while maintaining the others. Each sample was analysed for 3 min, by applying a desolvation temperature ramp and running a blank before and after each of the samples. Currently, ASAP requires manual sample loading and introduction. Both positive and negative ion modes were operated under atmospheric pressure ionisation (API) conditions to select the mode yielding the largest abundance of ions. The ASAP source temperature was set at 120 °C. The flow rate of the desolvation gas was 800 L/h. A scan time of 0.5 s and interval time of 10 s were applied over the m/z range 50–600 using leucine enkephalin as the LockSpray reference (556.2771). Instrument control, and data acquisition and handling were performed using MassLynx 4.1 software (Waters, Milford, MA, USA).

Prior to sample analysis, the source temperature was set to 150 °C and the desolvation gas temperature to 500 °C for 1 h to remove any residual contamination. Instrumental conditions, as optimised for the PPD standard, were used for the sample analyses. To prevent sample carry-over between subsequent samples, the glass capillary was inserted into the source chamber at 500 °C for at least 30 s to remove any contamination from the tip. The capillary rod was then removed, cooled and dipped into the next henna sample for ten seconds. Sample overload and contamination of the ion source was avoided by carefully removing excess solid from the capillary with tissue paper before inserting it into the ion source via the ASAP.

The limit of detection (LOD) was evaluated by spiking a blank henna sample with decreasing concentrations of PPD (2.0–0.025% w/w) and subsequently determining the PPD under the same conditions as the samples. The lowest concentration that yielded a

signal-to-noise (S/N) ratio of 3 for the PPD signal was regarded as the LOD.

2.4. UPLC–MS analysis

A Waters Acquity Ultra Performance Liquid Chromatographic system with photodiode array (PDA) detector (Waters, Milford, MA, USA) was used for the quantitative determination of PPD in the henna samples. Chromatographic conditions were optimised to obtain a well-resolved peak for PPD within a short analysis time. Separation was achieved on an ACQUITY UPLC BEH C₁₈ column (150 mm × 2.1 mm, i.d., 1.7 µm particle size, Waters), maintained at 30 °C. The mobile phase consisted of 0.1% formic acid in water (Solvent A) and acetonitrile (Solvent B) at a flow rate of 0.3 mL/min. Gradient elution was applied as follows: the initial concentration was 100% Solvent A, held for 2 min, then changed to 40% Solvent B over 4 min, then to 95% B within 2 min, maintained for 1 min, before returning the gradient to 100% Solvent A over 0.5 min and equilibrating the system for 2.5 min. The total run time was 12 min. A full-loop injection of 2.0 µL was done. The UPLC system was interfaced with a Xevo G2-Q-ToF-MS (Waters, USA). The same column, elution gradient and flow rates as before were used for the UPLC–MS analysis. Positive ion mode was used. Nitrogen was used as the desolvation gas at 420 °C and a flow rate of 500 L/h. The capillary and cone voltages were set to 3800 and 35 V, respectively. All the analyses were acquired by using the lock spray to ensure accuracy and reproducibility; leucine–enkephalin was used as the lock mass, generating an [M+H]⁺ ion at 556.2771 Da in ESI⁺ mode. Data were collected between *m/z* 50 and 600 and processed by MassLynx 4.1 chromatographic software (Waters).

Method validation was performed according to the ICH Harmonised Tripartite Guideline [23]. A calibration curve was constructed for PPD after diluting appropriate volumes of a freshly prepared stock solution in methanol to concentrations of 0.50, 1.0, 2.0, 5.0, 10, 25, 50 and 100 µg/mL. The LOD and lower limit of quantitation (LOQ) were established based on the S/N ratio approach, where LOD was expressed as S/N = 3 and LOQ as S/N = 10. Method precision was measured as the relative standard deviation (RSD) of the measured concentration of PPD (N = 3) to the theoretical concentration. The accuracy of the method was determined by determining the recovery from henna samples spiked at three concentration levels (2.0, 25 and 100 µg/mL) with PPD. In addition, the stability of PPD was evaluated by spiking henna samples (N = 3), containing no PPD, with a known concentration of PPD (100 µg/mL; Time 0) and re-analysing the samples at various time intervals (2, 4, 6, 8 and 24 h). The samples were kept at room temperature in a transparent vial exposed to daylight conditions. The PPD concentrations were calculated and the corresponding means were compared.

3. Results and discussion

3.1. Optimisation of the ionisation conditions for ASAP-Q-ToF-MS

Positive ion mode yielded the higher specific signal of PPD and was used throughout. For the MS spectrum, the characteristic mass (*m/z* = 109.0708) was selected and this information was used for investigating the presence or absence of PPD in the commercial samples. Various parameters i.e. cone voltage, corona current and desolvation gas temperature were optimised to improve detection sensitivity. The temperature of the desolvation gas is a crucial factor in analyte desorption and also influences the degradation of thermally labile compounds [16]. To optimise the desolvation temperature, the melting point of PPD (267 °C) was considered, since this temperature is normally the starting point for desolvation tem-

Table 1

p-Phenylenediamine detected by ASAP-MS, and quantified by UPLC-Q-ToF-MS, in henna samples from various countries.

Country	of origin	No of samples screened	Code of sample positive for PPD by ASAP-MS	PPD in sample (% w/w) by UPLC–MS
India		62	021	3.67
			075	3.91
			088	0.61
			100	1.75
			111	1.46
			115	0.05
			127	2.42
			149	0.81
			167	0.54
			–	N.d.
USA		9	–	N.d.
Egypt		6	–	N.d.
Pakistan		12	116	0.05
Iran		5	–	N.d.
Yemen		2	–	N.d.
Morocco		2	–	N.d.
France		1	–	N.d.
Sudan		2	–	N.d.
Mauritania		2	–	N.d.
Italy		1	–	N.d.
Japan		1	019	0.50
Turkey		1	–	N.d.
Russia		1	–	N.d.
Canada		1	–	N.d.
Nigeria		1	–	N.d.
Total samples positive			11	11

N.d.= not detected.

perature selection to ensure progressive and optimal desorption of the analyte. However, it was evident from the extracted ion trace, obtained by increasing the desorption temperature (Fig. 2), that PPD was already partially desorbed at 150 °C. As the temperature increased, decay of the ion current was observed that is consistent with depletion of the sample. An optimal desorption temperature of 150 °C was therefore selected.

A corona current of 5 µA and sample cone voltage of 30 V were selected, since these conditions yielded the most PPD ions. The mass spectrum of PPD obtained following subtraction of the background spectrum (Fig. 3) indicates the base peak at *m/z* 109.0708, corresponding to [M+H]⁺ for PPD, while the less abundant peak at *m/z* 92.0564 corresponds to [M–NH₂]⁺.

3.2. Screening of henna samples for PPD using ASAP-Q-ToF-MS

The extracted ion pseudo-chromatogram of a henna sample found positive for PPD by ASAP-MS is presented in Fig. 4a. According to the mass spectrum (Fig. 4b), two intense peaks at *m/z* 109.0714 and 92.0563 were observed that corresponded to the mass spectrum of the PPD standard, thereby confirming the presence of the compound in the sample. Eleven of the 109 samples were found to be positive for PPD (Table 1). The LOD value was determined as 0.025% (w/w).

3.3. Quantification of PPD in henna samples by UPLC–MS

In this study, a validated UPLC–MS method was used to confirm the results obtained from ASAP analysis and quantify the PPD in henna sample extracts. The total run time of the UPLC–MS was 12 min, which is much longer than the 3 min needed for ASAP (Fig. 5a). Since the PPD elutes early (*R_t* = 1.11 min), a short column wash could replace the gradient elution used. Analysis by UPLC resulted in improved resolution for the highly polar PPD when compared to that obtained by Almeida et al. [24] using conventional high performance liquid chromatography. The calibration

curve constructed from the analysis of the PPD calibration standards was found to be linear ($r^2 = 0.9981$) over the concentration range 0.50–100 $\mu\text{g/mL}$. The LOD and LOQ were determined as 0.44 and 1.34 $\mu\text{g/mL}$ respectively, while the intra-day precision value was 1.92%. The method accuracy varied from 95.8% to 99.7%. The stability evaluation indicated that PPD was stable for the first two hours, whereafter it reduced to 97.2% of the original concentration after 4 h, and then rapidly deteriorated to 44.0% after 24 h.

Of the 109 samples analysed, the same 11 samples found to be positive for PPD using ASAP were found to contain levels of PPD ranging from 0.05–4.21% (w/w) by UPLC–MS (Table 1). No PPD was detected in the remaining 98 samples when either technique was applied. Almeida et al. [24] determined PPD in three tattoo products and eleven henna hair dyes from five countries. They found that the PPD concentrations ranged from 1.17 to 64% in the tattoo dyes and from 2.09–12.8% in two of the hair dyes, while no PPD was detected in the remainder of the samples. The results of our study indicate that the ASAP technique, if applied to the samples of Almeida et al. [24], would have detected PPD in the positive samples, since the LOQ for the ASAP technique was established at 0.025% (w/w).

4. Conclusions

The globalisation of henna body art, and numerous injuries caused by painting skin with PPD as ‘black henna’ over the past twenty years has resulted in the outlawing of ‘black henna’ containing PPD in many countries [25], though black hair dyes with high concentrations of PPD as well as industrial PPD, remain readily available [26]. These supply sources are put into use to create ‘black henna’ temporary tattoos [24]. Since such tattoos are largely purveyed through informal economic activity, enforcement of these laws has been ineffective, and the popularity of painting the skin with PPD as ‘black henna’ has contributed to an epidemic of PPD-related sensitisation [27]. The availability of rapid methods to screen dyes and tattoo products will facilitate law enforcement.

This is the first report on the application of ASAP–Q–ToF–MS for the screening of henna samples to determine the presence of PPD. The suitability of the technique was confirmed by comparing the results with those from UPLC–Q–ToF–MS. Direct analysis of the solid sample prevents degradation in aqueous media and permits large numbers of samples to be screened, because no sample preparation (other than grinding) or chromatographic separation is involved. It was found that ASAP–Q–ToF–MS is a powerful, convenient, rapid, reliable and eco-friendly tool for the screening of henna products for PPD. Samples found to be positive for PPD using the probe can be further analysed by the more tedious UPLC–Q–ToF–MS technique to quantify the levels present. In this study, UPLC–Q–ToF–MS was successfully applied with minimal sample preparation, thereby preventing any degradation of PPD.

Ten percent of the samples analysed were found to contain PPD, indicating that henna products do pose a risk to consumers. Rapid detection of PPD using the ASAP probe may encourage screening of henna products, to protect individuals who are susceptible to the allergen.

Acknowledgements

The work was financially supported by the National Research Foundation, SAMRC Herbal Drugs Research Unit and the Tshwane University of Technology, South Africa. Caryn Hepburn and Tyren Dodgen (MICROSEP, South Africa) are thanked for valuable comments on the draft manuscript.

References

- [1] C. Cartwright-Jones, Developing Guidelines on Henna: A Geographical Approach (Masters' Dissertation). Masters of Liberal Studies, Kent State University, Kent, Ohio, USA, 2006, Available online at: www.hennapage.com/henna/.
- [2] I.M. Majoie, D.P. Bruynzeel, Occupational immediate-type hypersensitivity to henna in a hairdresser, *Am. J. Contact Dermat.* 7 (1996) 38–40.
- [3] F. Eghbal, M.R. Fakoorziba, M.H. Eghbal, S. Latifnia, Survey on causes of hemolysis in glucose-6-phosphate dehydrogenase (G6PD) deficient pediatric patients, *Pak. J. Med. Sci.* 24 (2012) 666–669.
- [4] D. Marzin, D. Kirkland, 2-Hydroxy-1,4-naphthoquinone, the natural dye of henna, is non-genotoxic in the mouse bone marrow micronucleus test and does not produce oxidative DNA damage in Chinese hamster ovary cells, *Mutat. Res.* 560 (2004) 41–47.
- [5] S. Hashim, Y. Hamza, B. Yahia, F. Khogali, G. Sulieman, Poisoning from henna dye and para-phenylenediamine mixtures in children in Khartoum, *Ann. Trop. Pediatr.* 12 (1992) 3–6.
- [6] S. Sharma, V. Dixit, P-Phenylenediamine and hair dyes, *IJPRD* 5 (2013) 95–102.
- [7] J.P. Johansen, D. Lepoittevin, J. Basketter, Allergens of special interest, in: P.J. Frosch, T. Menne, J.P. Lepoittevin (Eds.), *Contact Dermatitis*, 4th edn., Springer, Berlin, 2006, pp. 507–536.
- [8] A.M. Kligman, The identification of contact allergens by human assay. 3. The maximization test: a procedure for screening and rating contact sensitizers, *J. Invest. Dermatol.* 47 (1966) 393–409.
- [9] S.H. Wakelin, D. Creamer, R.J. Rycroft, I.R. White, J.P. McFadden, Contact dermatitis from paraphenylenediamine used as a skin paint, *Contact Dermat.* 39 (1998) 92–93.
- [10] J. Farrell, C. Jenkinson, S.N. Laverigne, J.L. Maggs, B.K. Park, D.J. Naisbitt, Investigation of the immunogenicity of p-phenylenediamine and Bandrowski's base in the mouse, *Toxicol. Lett.* 185 (2009) 153.
- [11] L.F. Corbett, Hair Colorants: Chemistry and Toxicity, Micelle Press, Weymouth, 1998.
- [12] M.L. Di Gioia, A. Leggio, A. Le Pera, A. Liguori, A. Napoli, F. Perri, C. Siciliano, Determination by gas chromatography/mass spectrometry of p-phenylenediamine in hair dyes after conversion to an imine derivative, *J. Chromatogr. A* 1066 (2005) 143–148.
- [13] K.M. Mohamed, D. Cromarty, V. Steenkamp, Development and validation of an LC–MS/MS method for determination of p-phenylenediamine and its metabolites in blood, *J. Chromatogr. B* 997 (2015) 1–6.
- [14] C.N. McEwen, R.G. McKay, B.S. Larsen, Analysis of solids liquids, and biological tissues using solids probe introduction at atmospheric pressure on commercial LC/MS instruments, *Anal. Chem.* 77 (2005) 7826–7831.
- [15] D. Mickael, D.P. Gaud, G. Audrey, P. Karinne, M. Fabrice, L.B. Bruno, High throughput identification and quantification of anabolic steroid esters by atmospheric solids analysis probe mass spectrometry for efficient screening of drug preparations, *Anal. Chem.* 86 (2014) 5649–5655.
- [16] C. Petucci, J. Diffendal, Atmospheric solids analysis probe: a rapid ionization technique for small molecule drugs, *J. Mass Spectrom.* 43 (2008) 1565–1568.
- [17] R.J. Fussell, D. Chan, M. Sharman, An assessment of atmospheric-pressure solids-analysis probes for the detection of chemicals in food, *Trends Anal. Chem.* 29 (2010) 1326–1335.
- [18] R.B. Cody, J.A. Laramée, D.H. Dupont, Versatile new ion source for the analysis of materials in open air under ambient conditions, *Anal. Chem.* 77 (2005) 2297–2302.
- [19] E.S. Chernetsova, G.E. Morlock, Determination of drugs and drug-like compounds in different samples with direct analysis in real time mass spectrometry, *Mass Spectrom. Rev.* 30 (2011) 875–883.
- [20] E.C. Horning, M.G. Horning, D.I. Carroll, I. Dzidic, R.N. Stillwell, New pictogram detection system based on MS with an external ionization source at atmospheric pressure, *Anal. Chem.* 45 (1973) 936–943.
- [21] D. Carrizo, C. Domeño, I. Nerín, P. Alfaro, C. Nerín, Atmospheric pressure solid analysis probe coupled to quadrupole-time of flight mass spectrometry as a tool for screening and semi-quantitative approach of polycyclic aromatic hydrocarbons, nitro-polycyclic aromatic hydrocarbons and oxo-polycyclic aromatic hydrocarbons in complex matrices, *Talanta* 131 (2015) 175–184.
- [22] Waters Atmospheric Solids Analysis Probe Documentation Bookshelf. Revision H, 2011. USA.
- [23] ICH Harmonised Tripartite Guideline. Validation of analytical procedures: Text and methodology. 1994 Available from ich.org/fileadmin/Public/WebSite/ICH_Products/Guidelines.
- [24] P.J. Almeida, L. Borrego, E. Pulido-Melián, O. González-Díaz, Quantification of p-phenylenediamine and 2-hydroxy-1,4-naphthoquinone in henna tattoos, *Contact Dermat.* 66 (2012) 33–37.
- [25] C. Cartwright-Jones, The geographies of the black henna meme organism and the epidemic of para-phenylenediamine sensitization: a qualitative history, in: PhD Thesis, Kent State University, Kent, Ohio, 2015, Available online at: www.hennapage.com/henna/.
- [26] E. Özkaya, K.D. Yazganoglu, A. Arda, Z. Topkarcı, E. Erçağ, The henna stone myth, *Indian J. Dermatol. Venereol. Leprol.* 79 (2013) 254–256.
- [27] S. Smith, Allergic contact dermatitis in children: trends in allergens, 10 years on. A retrospective study of 500 children tested between 2005 and 2014 in one U.K. centre, in: British Association of Dermatologists' Annual Conference, Leeds Teaching Hospitals NHS Trust, Leeds, U.K., 2015.