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A rapid method for chemical fingerprint analysis of *Hoodia* species, related genera, and dietary supplements using UPLC–UV–MS

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ABSTRACT

Recently, ultra-performance liquid chromatography (UPLC) has proven to be one of the most promising developments in the area of high-speed chromatographic separations with increased sensitivity and resolution. In this work, a reverse phase chromatographic method was developed using UPLC for the chemical fingerprint analysis of 12 hoodigosides, related genera and dietary supplements. The method is also used for the quantification of P57 in *Hoodia* species and dietary supplements that claim to contain *Hoodia*. The analysis was performed on a Waters Acquity UPLC system with an Acquity UPLC BEH C18 column (100 mm × 2.1 mm I.D., 1.7 μm) and a gradient elution of water and acetonitrile, both containing 0.05% formic acid with a run time of 15 min. The calibration curve of P57 showed good linearity ($r^2 > 0.999$) within the established range (1–100 μg/mL). The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.3 and 0.9 μg/mL, respectively. The RSD for intra- and inter-day were less than 3.0%, and the recovery efficiency as 97–103%. LC–mass spectrometry coupled with electrospray ionization (ESI) interface method is described for the identification of P57. The developed method was successfully applied to the identification of 12 oxypregnane glycosides in four different species of *Hoodia*, 23 related genera and 35 dietary supplements that claim to contain *H. gordonii*. The UPLC profiles of various plant samples were compared for the presence of oxypregnane glycosides. Different sample matrices were successfully analyzed, providing the wide range of applicability of this method, including gels, capsules, tablets, sprays, tea bags, snack bars, powders and juices.

1. Introduction

Hoodia gordonii is traditionally used by San tribe in South Africa for its appetite suppressant properties. There are 13 reported species (*H. alstonii*, *H. currorii*, *H. dregei*, *H. flava*, *H. gordonii*, *H. juttae*, *H. mossamedensis*, *H. officinalis*, *H. parviflora*, *H. pedicellata*, *H. pilifera*, *H. ruschii*, and *H. triebneri*) in the genus *Hoodia*; however, because of its anorectic activity *H. gordonii* is the only sought-after species for trade [1–3]. *H. gordonii* is currently listed as an endangered species and its export out of South Africa is strictly controlled by the South African government and CITES. At the same time, the demand for weight loss products containing *H. gordonii* is increasing and there are over 100 products currently marketed in the US in the following dosage forms: tablet, capsule, liquid gels, liquid tinctures, snack (or fruit) bars, juice, *Hoodia* powders, protein shakes, *Hoodia* lollipops, *Hoodia* tea and coffee. Because the supply of authentic *H. gordonii* cannot match the demand for *Hoodia* products, the adulteration of the product by other species or even other genera then becomes a possibility.

H. gordonii is a rich source of pregnane glycosides. P57, an oxypregnane steroidal glycoside, is the only reported active constituent from this plant that acts as an appetite suppressant [1]. The aglycone was characterized as 12-*O*-β-tigloyl-3β, 14β-dihydroxy-pregn-5-en-20-one, which was named as hoodigogenin A. The sugar chain located at C-3 was identified as 3-*O*-β-D-thevetopyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-cymaropyranoside.

A high-performance liquid chromatographic method for the determination of P57 in *Hoodia* species and dietary supplements by LC–ESI–TOF and LC–UV methods was established by Avula et al., [2]. Avula et al., [3] also reported the separation of 11 steroidal glycosides and chemical fingerprint analysis by high-performance liquid chromatography (HPLC). These previously developed methods can be used for the authentication of *Hoodia* plant sample but

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require long run times. In order to reduce the run time and increase resolution, we decided to investigate the use of ultra-performance liquid chromatography (UPLC) technology. In the present work, UPLC–UV/MS methods have been developed for the analysis of twelve steroidal glycosides, which are used for a chemical fingerprint analysis and quantitative determination of P57 in various species of *Hoodia* and dietary supplements claiming to contain *H. gordonii*. A mass spectrometry coupled with electrospray ionization (ESI) interface method is described for the confirmation of P57 in various *Hoodia* species and dietary supplements.

Adulteration of products claiming to contain *H. gordonii* is possible; developing techniques for rapid product analysis becomes crucial for product validity and safety. The present analytical method provides a fast determination tool for authentication of *H. gordonii* with a combined chemical fingerprint analysis and the confirmation of the marker compound, P57.

UPLC makes it possible to perform high-resolution separations in short periods of time with little solvent consumption [4–8], utilizing solid phase particles of 1.7 μm diameter to achieve superior theoretical plates and resolution.

2. Materials and methods

2.1. Instrumentation and chromatographic conditions

All analyses were performed on a Waters Acquity UPLC system (Waters Corp., Milford, MA, USA) including binary solvent manager, sampler manager, column compartment and PDA detector, connected to a Waters Empower 2 data station. An Acquity UPLC BEH C18 column (100 mm × 2.1 mm I.D., 1.7 μm) also from Waters was used. The column and sample temperature were maintained at 40 °C and 20 °C, respectively. The column was equipped with a LC-18 guard column (Vanguard 2.1 mm × 5 mm Waters Corp., Milford, MA, USA). The mobile phase consisted of water with 0.05% formic acid (A), and acetonitrile with 0.05% formic acid (B) at a flow rate of 0.35 mL/min, with gradient elution as follows: 0 min, 70% A/30% B in next 15 min to 30% A/70% B and finally, reconditioning the column with 70% A/30% B for 3.5 min after washing column with 100% B for 2 min. The composition of mobile phase was changed linearly (Waters curve type 6). The total run time for analysis was 15 min. The injection volume was 5 μL. The peaks were detected using UV at 220 nm.

The effluent from the LC column was directed into the ESI probe. Mass spectrometer conditions were optimized to obtain maximal sensitivity. The source temperature and the desolvation temperature were maintained at 150 °C and 350 °C, respectively. The probe voltage (capillary voltage), cone voltage and extractor voltage were fixed at 1.5 kV, 65 V and 2 V, respectively. Nitrogen was used as the source of desolvation gas (650 L/h) and drying gas (25 L/h). P57 was confirmed in selected ion recording (SIR) mode. $[M + Na]^+ = 901.6$ ion for P57 was selected as detecting ion. Mass spectra were obtained at a dwell time of 0.1 s in SIR and 5000 Da/s of scan rate.

2.2. Chemicals

The standard compounds [hoodigoside K (1), hoodigoside L (2), hoodigoside O (3), hoodigoside P (4), hoodigoside E (5), hoodigoside F (6), hoodigoside J (7), hoodigoside S (8), P57 (9), hoodigoside U (10), hoodigoside I (11), and hoodigoside C (12) (Fig. 1)] were isolated at the NCNPR, the identity and purity was confirmed by chromatographic (TLC, HPLC) methods, by the analysis of the spectroscopic data (IR, 1D- and 2D-NMR, HR–ESI–MS) and comparison with published spectroscopic data [9,10].

Acetonitrile, water and formic acid were of HPLC grade, purchased from Fisher Scientific (Fair Lawn, NJ, USA).

Different *Hoodia* species and various related plant samples; *Hoodia ruschii* (Voucher # 2822), *Hoodia currorii* (Voucher # 2823), *Asclepias labriformis* (Voucher # 2614), *Huernia keniensis* Apocynaceae member (Voucher # 2818), *Tridentea choanantha* (Voucher # 2813), *Stapelia grandiflora* (Voucher # 2812), *Edithcolea grandis* (Voucher # 2815), *Cynanchum marnierianum* (Voucher # 2825), *Heurnia recondita* (Voucher # 2819), *Stapelia gigantea* (Voucher # 2809), *Stapelia schinzii* (Voucher # 2810), *Stapelia leendertziae* (Voucher # 2811), *Piaranthus globosus* (Voucher # 2814), *Cynanchum perrieri* (Voucher # 2824), *Ceropegia dicotoma* (Voucher # 2820), *Orbea variegata* (Voucher # 2817) were obtained from Missouri Botanical Garden, *Hoodia parviflora* (Voucher # 3163), was obtained from AHP, *Gonolobus cundurango* (Voucher # 1738), *Marsdenia cundurango* (Voucher # 1598) are in-house NCNPR samples, *Caralluma fimbriata* (Voucher # 2971) was procured from Natures Sunshine, USA. *Hemidesmus indicus* (Voucher # 1224) and *Gymnema sylvestris* (Voucher # 1223) were obtained from Hyderabad, India, *Opuntia ficus-indica* (Voucher # 2888) was purchased from local stores. *Cynanchum stratum* (Voucher # 838), *Cynanchum stauntonii* (Voucher # 780) were obtained from Beijing Yuke Botanical Development Co. Ltd., China. *Vincetoxicum hirundinaria* (Voucher # 42) was obtained from Italy. *H. gordonii* (Voucher # 2926) was obtained from Universiteit Van Die Vrystaat, South Africa. Voucher specimens of all samples are deposited at the National Center for Natural Products Research (NCNPR), University of Mississippi, Mississippi, USA.

2.3. Sample preparation

The dietary supplements analyzed in this work were in multiple dosage forms including: tablets/capsules/tea bags/powders/liquid gels/juices/sprays/snack bars. In order to perform the determinations on these different matrices we developed an extraction protocol specific for each class of formulation.

2.3.1. For tablets/snack bars/capsules/tea bags/powders

For tablets, five tablets were weighed and then pulverized with a mortar and pestle. For capsules/tea bags/powder packets, five samples were weighed, opened and the contents were emptied, then mixed and triturated in a mortar and pestle.

Dry plant samples (0.3 g) or an adequate amount of powdered tablet/snack bars (2 g) or capsule/tea bags/powder pack contents were weighed (average weight of dosage form) and sonicated in 2–4 mL of methanol for 20 min followed by centrifugation for 15 min at 4000 rpm. The supernatant was transferred to a 10 mL volumetric flask. The procedure was repeated thrice and the respective supernatants combined. The final volume was adjusted to 10 mL with methanol and mixed thoroughly.

Prior to injection, an adequate volume (ca. 2 mL) was passed through a 0.2 μm nylon membrane filter. The first 1.0 mL was discarded and the remaining volume was collected in an LC sample vial. Each sample solution was injected in triplicate.

2.3.2. For liquid gels

Liquid/soft gels are oily in nature; hence to improve the extraction efficiency the method was modified.

Adequate amount of content of soft gels (average weight of five soft gels) were sonicated in 0.5 mL of hexane and 2.5 mL of methanol for 20 min followed by centrifugation for 15 min at 4000 rpm. The supernatant was then processed as in section 2.3.1.

2.3.3. For juices/sprays

1 mL of juice/spray solution was mixed with 1.0 mL of methanol, vortex for 30 s and sonicated for 30 min, vortexed and centrifuged for 15 min at 4000 rpm. The clear supernatant solution was used for analysis.

2.4. Preparation of standard solution including P57

An individual stock solution of the standard compounds was prepared at a concentration of 1.0 mg/mL in methanol. 50 μL of each standard solution was mixed together and the mixture was further diluted to a concentration of 15 μg/mL which was used for UPLC analysis.

2.4.1. Standard solutions of P57

A stock solution of standard compound (P57) was prepared at a concentration of 1.0 mg/mL in methanol. The calibration curve was prepared at five different concentration levels. The range of the calibration curve was 1.0–100 μg/mL for UPLC–UV analysis.

2.5. Validation procedure

The newly developed UPLC method was validated in terms of precision, accuracy, and linearity according to ICH guidelines [11]. Assay method precision was carried out using five independent test and standard solutions. The accuracy of the assay method was evaluated in triplicate using two concentration levels of 5 and 10 μg/ml. The limit of detection (LOD) and limit of quantification (LOQ) were determined by injecting a series of dilute solutions with known concentrations. LOD and LOQ were defined as the signal-to-noise ratio equal to 3 and 10, respectively.

3. Results and discussion

3.1. Chromatographic conditions optimization

Optimal chromatographic conditions were obtained after running different mobile phases with a reversed phase C18 column. The different columns tried were Acquity UPLC BEH C18 (100 mm × 2.1 mm I.D., 1.7 μm), Acquity UPLC BEH C18

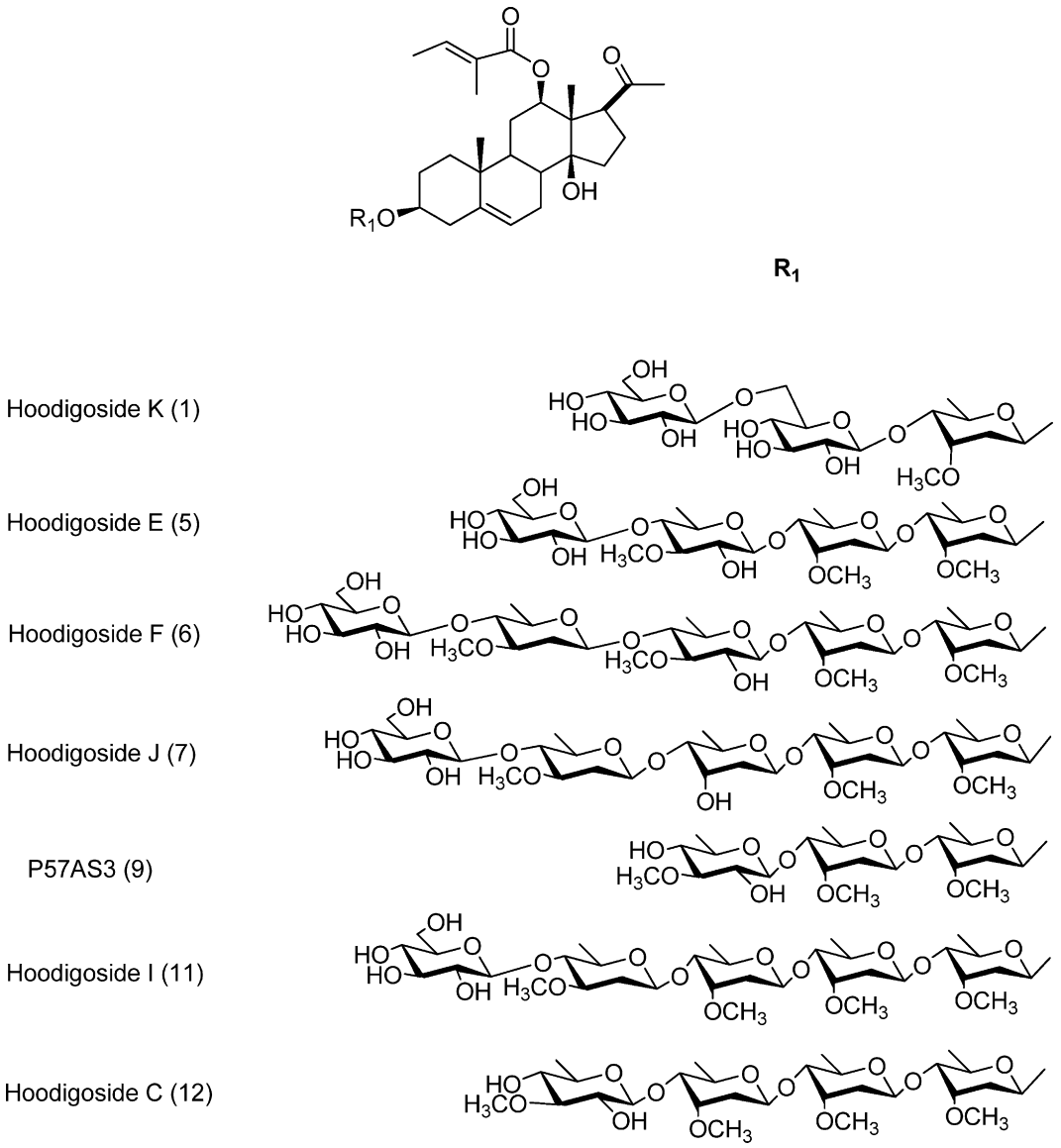

Fig. 1. Structure of steroidal glycosides.

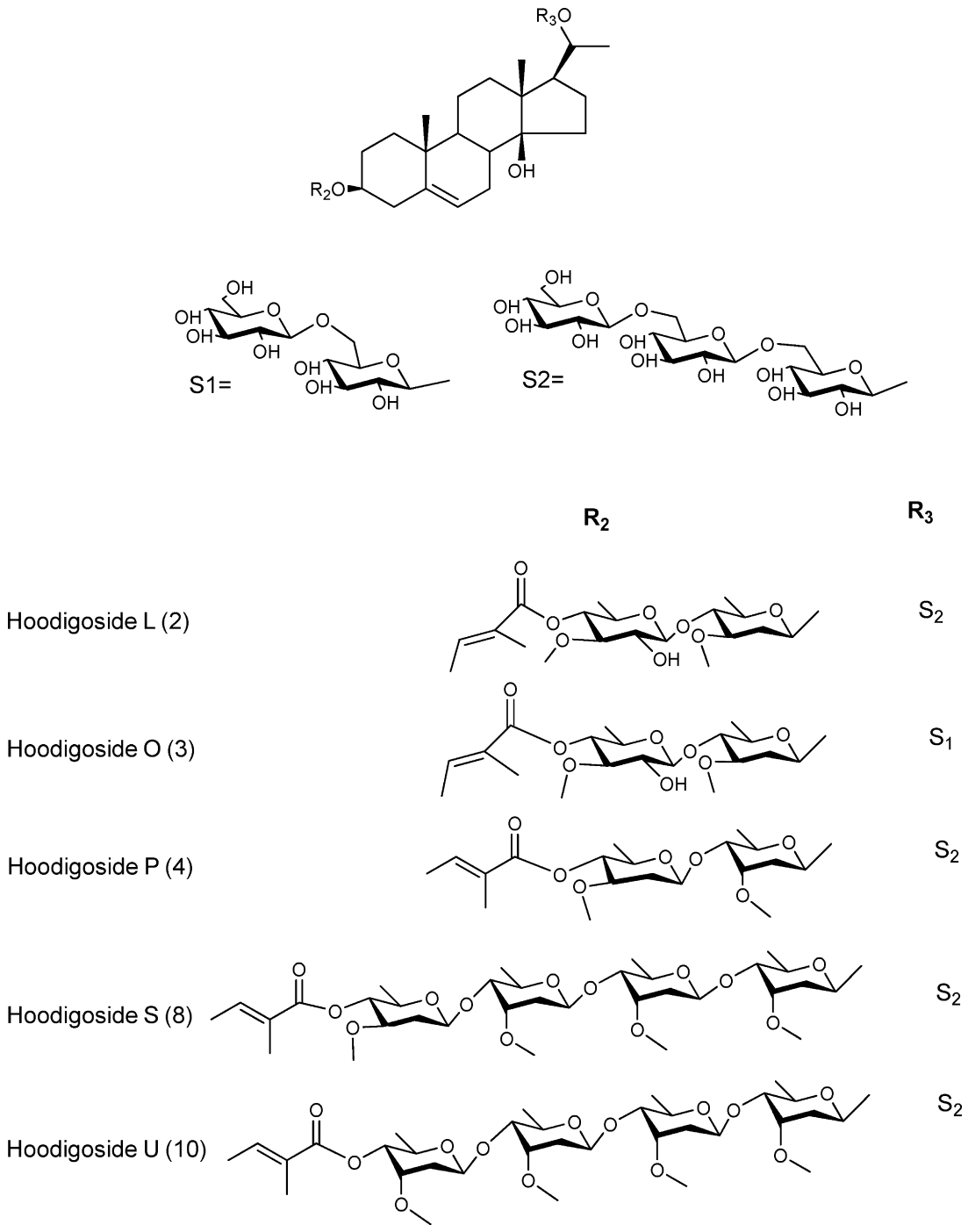

Fig. 1. (*Continued*).

(50 mm × 2.1 mm I.D., 1.7 μm) and Acquity UPLC BEH Shield RP18. The best results were observed with BEH C18 column (100 mm × 2.1 mm I.D., 1.7 μm) using water and acetonitrile, both containing 0.05% formic acid as the mobile phase. Acetonitrile was preferred over methanol as the mobile phase as its use resulted in improved separation as well as a significantly reduced column back pressure. The addition of a modifier like formic acid was advantageous for peak separation. Many different gradient

Table 1
Intra- and Inter-day precision for P57

Analyte	Concentration (μg/mL)	Intra-day (n = 5)			Inter-day (n = 15)		
		Found (μg/mL)	RSD (%)	Accuracy* (%)	Found (μg/mL)	RSD (%)	Accuracy (%)
P57	5.0	4.89	2.03	97.8	4.91	2.45	98.2
	10.0	10.23	2.76	102.3	9.89	2.67	98.9

* Accuracy (%) = 100% × mean of measured concentration/nominal concentration.

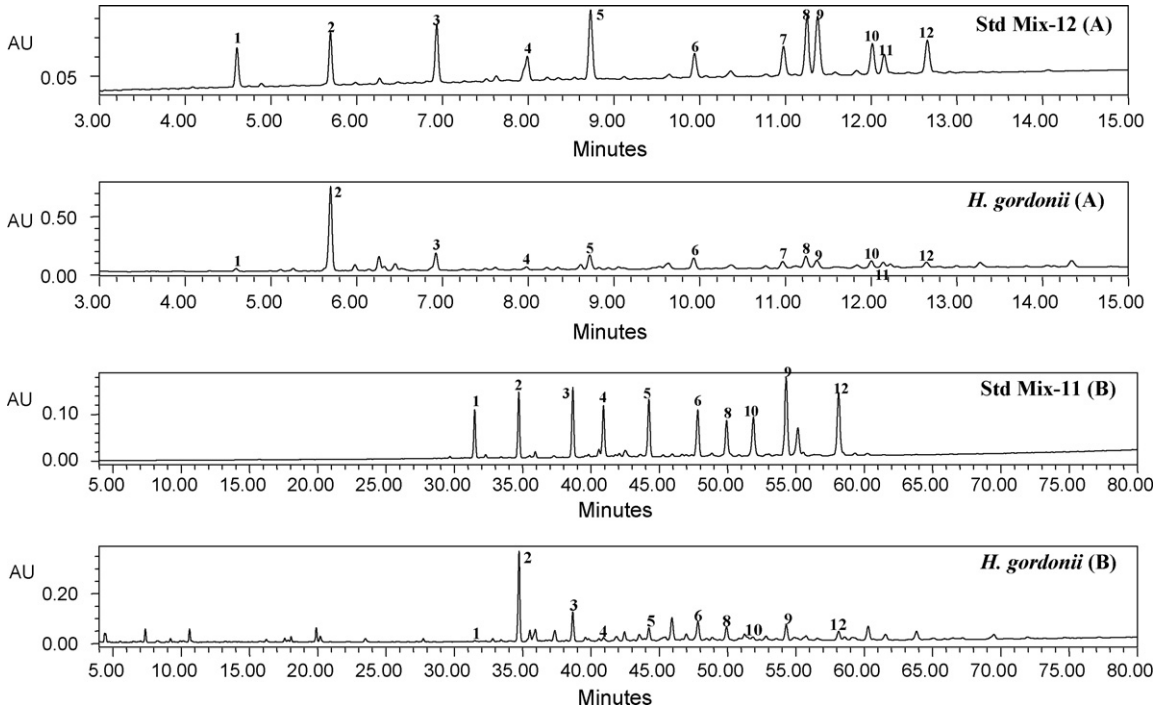

Fig. 2. Comparison of chromatograms of chemical fingerprinting obtained from (A) UPLC and (B) HPLC at wavelength 220 nm: (1) hoodigoside K; (2) hoodigoside L; (3) hoodigoside O; (4) hoodigoside P; (5) hoodigoside E; (6) hoodigoside F; (7) hoodigoside J; (8) hoodigoside S; (9) P57; (10) hoodigoside U; (11) hoodigoside I and (12) hoodigoside C.

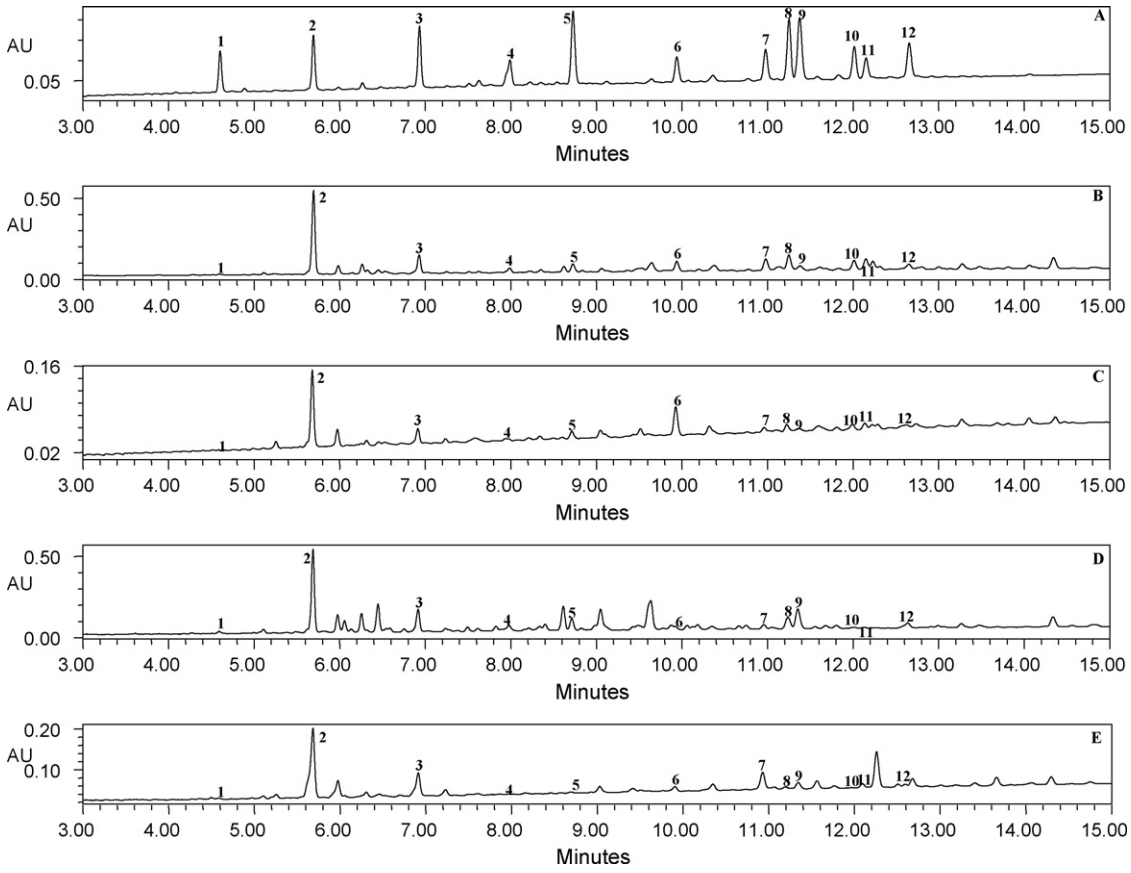

Fig. 3. Typical UPLC chromatograms of (A) mixed standards (12) and methanolic extracts of (B) *H. gordonii*; (C) *H. currorii*; (D) *H. ruschii*; (E) *H. parviflora*: (1) hoodigoside K; (2) hoodigoside L; (3) hoodigoside O; (4) hoodigoside P; (5) hoodigoside E; (6) hoodigoside F; (7) hoodigoside J; (8) hoodigoside S; (9) P57; (10) hoodigoside U; (11) hoodigoside I and (12) hoodigoside C at wavelength 220 nm.

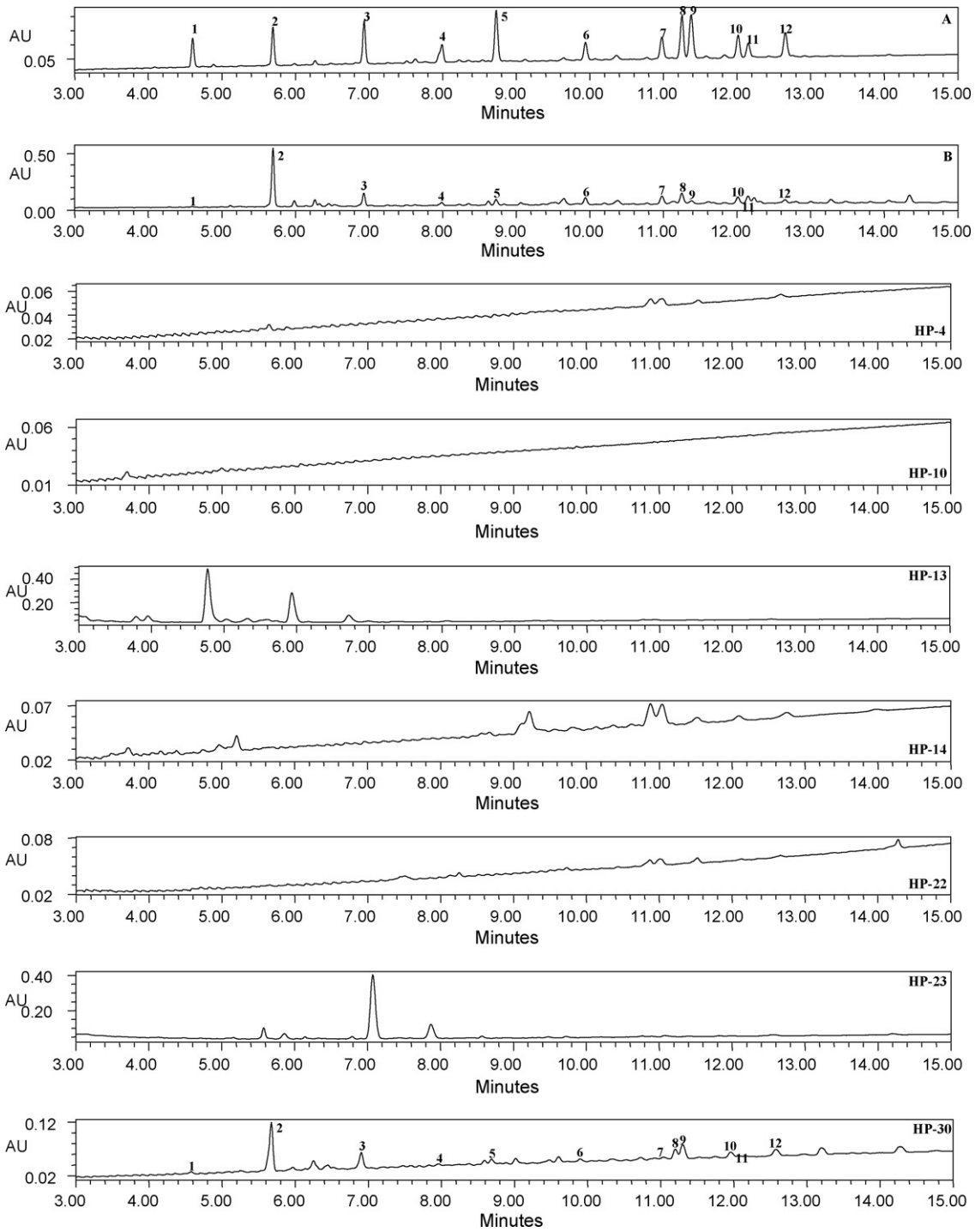

Fig. 4. Comparison of HPLC profiles of mixed standards (A); *H. gordonii* (B) and dietary supplements (HP-4, HP-10, HP-13, HP-14, HP-22, HP-23, HP-30) claiming to contain *H. gordonii* at wavelength 220 nm: (1) hoodigoside K; (2) hoodigoside L; (3) hoodigoside O; (4) hoodigoside P; (5) hoodigoside E; (6) hoodigoside F; (7) hoodigoside J; (8) hoodigoside S; (9) P57; (10) hoodigoside U; (11) hoodigoside I and (12) hoodigoside C.

systems of mobile phase were tried for the best separation of peaks.

3.2. Comparison study of chromatographic performance

A comparison of chromatographic performance of HPLC (gradient) and UPLC (gradient) was preformed and the run time for 12 pregnane glycosides was about 15 min in the UPLC system and 80 min in the HPLC system. The UPLC system allowed shortening the analysis time up to 5-fold compared to that of gradient mode HPLC system using 5 μm particle packed analytical columns. The typical chromatograms obtained from final HPLC and UPLC conditions of chemical fingerprint analysis are depicted in Fig. 2.

3.3. UPLC method validation

The validation study allowed the evaluation of the method for its suitability for routine analysis.

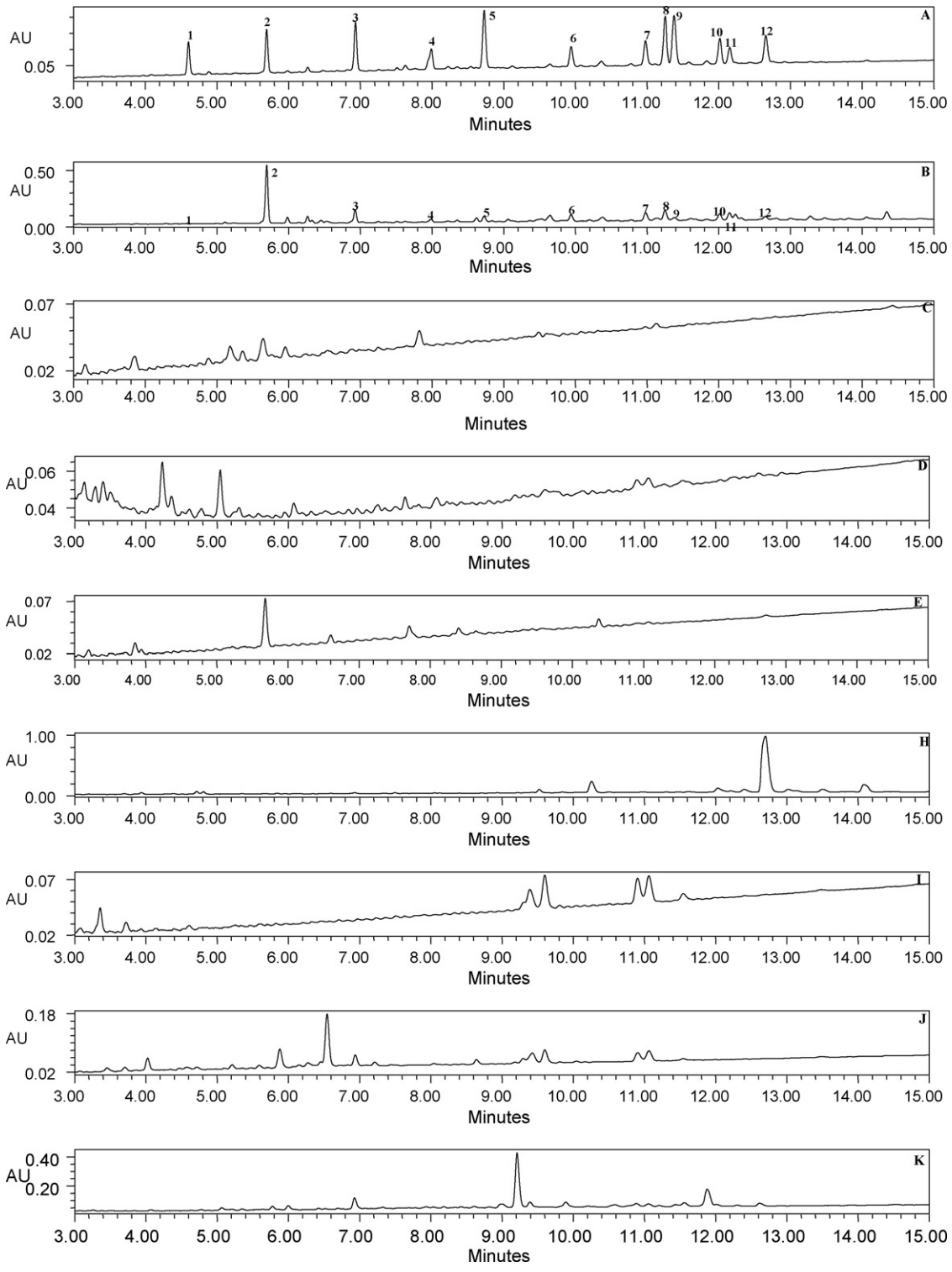

Fig. 5. Comparison of UPLC profiles of mixed standards (A); *Hoodia gordonii* (B); *Opuntia ficus-indica* (C); *Caralluma fimbriata* (D); *Edithcolea grandis* (E); *Tridentea choanantha* (H); *Asdepias labriformis* (I); *Gymnema sylvestris* (J); *Gonolobus cundurango* (K) at wavelength 220 nm.

3.3.1. Specificity

The specificity of the UPLC method was determined by injecting individual samples, wherein no interference was observed for any of the components. The chromatograms were checked for the appearance of any extra peaks. The purity of the principle and other chromatographic peaks was found to be satisfactory.

3.3.2. Precision

The precision of the assay method was evaluated by carrying out five independent assays. The % RSD of assay of P57 determination was within the acceptable limit of 3.0%. Multiple injections showed that the results are highly reproducible and showed a low standard error. The RSD of assay results obtained in inter-day and intra-day study (Table 1) was within 3.0% and the RSD with a maximum of

2.76% for P57, confirming a good precision of the developed method. It was performed five times on three different days and each sample was injected in triplicate.

3.3.3. Accuracy

The accuracy of the method was determined for the related substance by spiking samples with a known amount of P57. The accuracy of the assay method was evaluated in triplicate at two concentration levels, 5 and 10 μg/ml of P57 in the sample. The percentage recovery of P57 in samples ranged from 97.0% to 103.0%.

3.3.4. Limit of quantification and limit of detection

The limits of detection and limits of quantification for P57 were found to be 0.3 μg/mL and 0.9 μg/mL, respectively. LOD and LOQ were defined as the signal-to-noise ratio equal to 3 and 10, respectively. All standards and samples were injected in triplicate.

3.3.5. Linearity

Linear calibration plots for the related substance method were obtained over the calibration range at five concentration levels (1–100 μg/mL) in triplicate. Linear regression analysis of the calibration plot of P57 gave the equation $Y = 1.62e + 004X + 6.13e + 003$ and calibration data indicated the linearity (r^2>0.999) of the detector response for P57 by UPLC–UV method. The results showed excellent correlation between the peak area and concentration. The five point calibration curve for P57 showed a linear correlation between concentration and peak area.

3.3.6. Analysis of plant samples and dietary supplements

The LC–UV data (Figs. 3–5) and the LC–MS chromatograms (Fig. 6) show the presence or absence of P57 in *H. gordonii* plant sample (HG-1), related genera, and dietary supplements. The UPLC–UV method was applied for quantification of P57 in various plant samples and commercial products. The plant extracts and commercial products (HP-1–HP-35) were analyzed using LC–UV and LC–MS and the concentration of P57 by UPLC–UV method is shown in Tables 2 and 3. The content of P57 detected in HG-1, *H. ruschii*, *H. currorii* and *H. parviflora* were 0.043%, 0.254%, 0.218% and 0.056%, respectively (LC–UV), and was not detected in related genera. Unlike the crude plant material, *Hoodia* supplements are a mixture of *Hoodia* or *H. gordonii* extract with other botanicals (green tea leaf extract, cocoa extract, green coffee bean extract, cortinine blend, guarana extract, *Gymnema sylvestres*, *Panax ginseng*, yerba mate, *Ginkgo biloba*, ginger root, bee pollen, theobromine, grapefruit, white tea, black tea extract, oolong tea extract, rooibos tea extract, *Citrus aurantium*, white whillow bark extract, cola nut extract, *Garcinia cambogia*, dandelion root powder, spirulina, *Rhodeola rosea* extract, *Cissus quadrangularis* extract, tarragon extract, soy phospholipids, *Withania somnifera* extract, cinnamon bark extract, *trans*-resveratrol, glucomannon, natural lemon etc.) along with vitamins, minerals, excipients etc. in the following dosage forms: tablet, capsule, liquid gels, suspensions, snack bars, juice, tea bags or powder. Fig. 3, shows the UPLC–UV chromatograms of *H. gordonii* (B) and seven commercial products (C-I). Table 2 shows the % content of P57 in *Hoodia* species. Table 3 shows the different nature of information contained on the labels for the *Hoodia* supplements studied as compared to the mg/average weight of dosage form content of P57. Thirty-five commercially available dietary supplements were tested (HP-1–HP-35). Nine products contained P57. HP-1, HP-30 and HP-34 contained 0.016 mg/average weight of dosage form, 1.201 mg/average weight of dosage form and 54 μg/mL of P57, respectively, the other five (HP-7, HP-19, HP-22–HP-24) contained very low amounts of P57 which were detected under limits of quantification, and product HP-21, did not show the presence of P57 by UPLC–UV method but showed for its presence by UPLC–MS method. Twenty-six products (HP-2–HP-6, HP-8–HP-18, HP-20, HP-25–HP-29, HP-31–HP-33, HP-35) did not show the presence of P57 by either methods

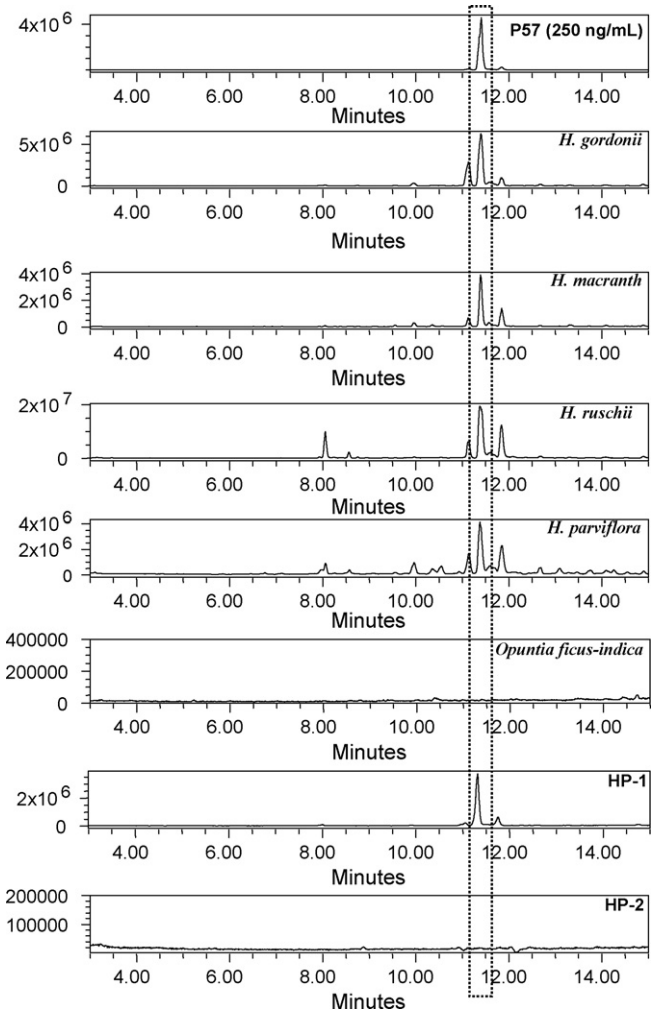

Fig. 6. Typical UPLC–MS chromatograms of P57, *Hoodia* species and dietary supplements (HP-1, HP-2) claiming to contain *Hoodia gordonii*.

Table 2
P57 content (%) in various *Hoodia* species by UPLC–UV & confirmation by UPLC–MS

	Species	P57 content (%) (mg/100 mg dried material)	
		UPLC–UV	UPLC–MS
Plants samples	*H. gordonii* (HG)	0.043	+
	H. ruschii (HR)	0.254	+
	H. currorii (HC)	0.218	+
	H. parviflora (HP)	0.056	+

Table 3
Content (mg) of P57 found in different *Hoodia* products and comparison of UPLC–UV data to information provided on *H. gordonii* product labels

Product code	Aveage. weight taken (mg)	Serving size	*H. gordonii* content/serving size	P57	
				UPLC–UV (mg/aveage weight of product)	UPLC–MS
HP-1	1405.9/tablet	1	–	0.0158	+
HP-2	549.7/capsule content	1	500 mg	ND	ND
HP-3	549.7/gel content	2	500 mg	ND	ND
HP-4	583.2/capsule content	1	400 mg	ND	ND
HP-5	1168.7/tablet	1	800 mg	ND	ND
HP-6	1 mL	1 mL	–	ND	ND
HP-7	706.6/capsule content	2	1000 mg	DUL	+
HP-8	1001.2/gel content	2	–	ND	ND
HP-9	983.3/capsule content	2	200 mg	ND	ND
HP-10	2 mL	120 mL	175 mg	ND	ND
HP-11	793.7/capsule content	2	–	ND	ND
HP-12	714.7/capsule content	1–2	–	ND	ND
HP-13	780.4/capsule content	1	–	ND	ND
HP-14	461.4/capsule content	2	200 mg	ND	ND
HP-15	1520.3/tablet content	2	–	ND	ND
HP-16	490.7/capsule content	2	200 mg	ND	ND
HP-17	688.2/capsule content	1	100 mg	ND	ND
HP-18	580.4/capsule content	1	–	ND	ND
HP-19	2093.7/tea bag content	1	–	DUL	+
HP-20	1259.2/tablet	1	200 mg	ND	ND
HP-21	3013.2/packet content	1	–	ND	+
HP-22	952.7/tablet	3	550 mg	DUL	+
HP-23	878.6/gel content	2	–	DUL	+
HP-24	742.2/gel content	2	550 mg	DUL	+
HP-25	1002.0/tablet	1	–	ND	ND
HP-26	2020.3 mg	–	–	ND	ND
HP-27	1119.9/tablet	1	1000 mg	ND	ND
HP-28	514.1/capsule content	1	400 mg	ND	ND
HP-29	233.9/capsule content	1	250 mg	ND	ND
HP-30	714.5/capsule content	1	750 mg	1.2058	+
HP-31	564.6/capsule content	2	200 mg	ND	ND
HP-32	871.8/tablet	3	–	ND	ND
HP-33	1229.0/tablet	1	500 mg	ND	ND
HP-34	1 mL	1 mL = 30 drops	250 mg/mL	54 μg/mL	+
HP-35	201.2/tablet	2	200 mg	ND	ND

ND = not detected; DUL = detected under limit of quantitation; '+' = detected.

(UPLC–MS and UPLC–UV). For some of the samples of related genera and dietary supplements displayed peaks with same RT as one or more of the 12 compounds. These peaks showed different UV-spectrum and also further confirmation of P57 was done by MS spectrometry. The twelve compounds including P57 were not detected in all the samples of non-*Hoodia* related genera analyzed. The UPLC–MS method (LOD = 1 ng/mL) was shown to be a more sensitive analytical method than the UPLC–UV method (300 ng/mL).

The UPLC–SQD analysis (Fig. 6) revealed that P57 had a molecular peak at 901.6 $[M+Na]^+$. No interfering peaks were found at the retention time of interest. By the LC–UV method, the identification of the P57 in *H. gordonii* samples and dietary supplements was based on the retention times and the comparison of UV spectra or by spiking the extracts with the reference compound, P57.

4. Conclusion

The newly developed UPLC method for chemical analysis of 12 hoodigosides was found to be capable of giving shorter retention times while maintaining good resolution than that compared to conventional HPLC. The method is suitable for rapid analysis of P57 and chemical fingerprint analysis. The developed method was validated for P57 for all the parameters tested and successfully applied to the identification of 12 oxypregnane glycosides in four different species of *Hoodia*, 23 related genera and 35 dietary supplements which claim to contain *H. gordonii*. The UPLC profiles of various plant samples were compared for the presence of oxypregnane glycosides. All four species of *Hoodia* and nine dietary supplements were found to contain P57. Different sample matrices were successfully analyzed, providing the wide range of applicability of this method, including gels, capsules, tablets, sprays, tea bags, snack bars, powders and juices. LC–mass spectrometry coupled with ESI method is described for the identification of P57 in various plant samples. This method involved the use of the $[M+Na]^+$ ions of P57 in the positive ion mode with selective ion recording (SIR).

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