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A stability-indicating high-performance liquid chromatographic assay for nicotine in transdermal patches

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Summary

This report describes a rapid, precise, stability-indicating method to analyze nicotine as a raw material or in transdermal dosage forms using high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection. The method uses a new multi-phase polymeric column which allows separation by a combination of reversed-phase and ion-exchange chromatography. The method was characterized with respect to specificity, linearity, accuracy, and precision.

Introduction

Transdermal delivery of nicotine into the systemic circulation has been shown to satisfy the physical craving for nicotine by providing a noninhaled form of the drug (Rose et al., 1985; Chan et al., 1990). This type of dosage form has the capability of maintaining constant low levels of nicotine in the blood over an extended period of time. Smoking cessation programs have been proposed consisting of a gradual reduction in the dosage of the drug being administered, coupled with psychological counseling (Krumpe et al., 1989).

Several analytical methods for the determination of nicotine in plasma, saliva, and urine have previously been reported, but were found to be inadequate for the analysis of nicotine transdermal patches. Analytical methods employing gas chromatography (Hengen and Hengen, 1978; Jacob et al., 1981) suffer the disadvantage of the inability to quantitate labile compounds, such as nicotine 1′-*N*-oxide, one of the metabolites of nicotine (Thompson et al., 1985). Several analytical methods employing HPLC are also described in the literature (Carmella and Hecht, 1985; Mousa et al., 1985; Barlow et al., 1987; Harlharan et al., 1988; Hefner et al., 1988). The two types of separation techniques discussed in these reports involve either ion pairing or ion suppression modes of HPLC (Meyer, 1988; Szepesi, 1990).

An HPLC method incorporating ion pairing is often chosen for the separation of weakly basic drugs (Snyder et al., 1988; Szepesi, 1991) such as nicotine in an effort to control the effects of ionization. Use of an ion pairing reagent precludes separation of the analytes based on their

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ionic properties; the most significant problem encountered with this technique is that it has inherently poor selectivity. Band tailing can be induced by inadequate buffering of the aqueous phase or dissociation of ion pairs in the organic phase (Knox and Jurand, 1975).

Ion suppression is another HPLC technique used to accomplish separation of weak bases (Snyder et al., 1988; Szepesi, 1991). The mobile phase is buffered at a particular pH to suppress any ionization, ensuring that only unionized solutes are available for partitioning, and retention is based on the adsorptive properties of the neutral analytes. In most cases, however, the pH required for full suppression is outside the stable range of columns with silica-based packings. The uncontrolled interaction of weak bases such as nicotine with residual silanol groups (-SiOH) on the surface of the packing often results in severe peak tailing and low column efficiency (Karger and Sibly, 1973).

The methods indicated above were developed for bioanalysis, i.e., the determination of nicotine and/or nicotine metabolites in biological fluids in support of pharmacokinetic studies. Although such methods are designed for high specificity, the high senstivity required for determination of very low concentration tends to result in assay methods that are not highly accurate or precise, as would ordinarily be desired for drug substance or drug product assays.

This report describes the development and characterization of a rapid, precise, stability-indicating method to analyze nicotine as a raw material in transdermal dosage forms, using high-performance liquid chromatography with ultraviolet detection. The method uses a new multi-phase polymeric column (Slingsby and Rey, 1990; LaCourse et al., 1991) which allows separation by a combination of reversed-phase and ion-exchange chromatography. The column packing consists of microporous resin beads whose outer surfaces have been permanently coated with a pellicular ion-exchange latex. The highly cross-linked, macroporous resin particles have a large enough surface area (300 m^2/g) to allow retention by a reversed-phase mechanism. The sulfonic acid-type cation-exchange sites of the latex coating can associate with either a cationic analyte or the eluting ion, allowing retention by a cation-exchange mechanism.

Materials and Methods

Chemicals

Nicotine salicylate was employed as a reference material and was obtained from Pfaltz and Bauer Co. (lot 37194); it was used without further purification. The purity of this material was evaluated prior to use by a combination of techniques including thermal and chromatographic methods. Differential scanning calorimetry with subsequent Van't Hoff analysis provided a major indication of purity. Two complementary stability-indicating thin-layer chromatographic methods provided further evidence of purity. The material was also analyzed employing the method described in this report, and was determined to be pure. Elemental analysis of the substance provided information regarding both structure and purity. Experiments for the determination of loss on drying and hygroscopicity were also performed. The nicotine salicylate used in these experiments was estimated to be not less than 99.94% pure (Wygant and Carlisle, unpublished observations). The material was protected from light and kept in a well-closed amber glass container.

Nicotine free-base was obtained from Kodak Chemicals (Kodak lot no. 8835009063) and was used without further purification. The purity of this material was based on HPLC determination, and was estimated to contain not less than 99.3% nicotine. The material was protected from light and kept in a well-closed amber glass container.

Authentic samples of nornicotine and cotinine were obtained from Sigma Chemicals (lot nos 118F0508 and 38F-110, respectively). Authentic samples of nicotine 1-*N*-oxide, nicotine 1'-*N*-oxide, and nicotine 1,1'-di(*N*-oxide) were provided by Recordati Industria e Farmaceutica S.p.A. (Milan, Italy).

Nicotine transdermal patches used in the experiments described in this report had nominal contents of 37 mg/5 cm^2. Placebo patches were made of the same materials as the active patches

but did not contain any nicotine. Both lots were manufactured at Pharmetrix Corp., Menlo Park, CA.

The acetonitrile used for the HPLC analyses was distilled-in-glass HPLC grade (Burdick and Jackson, Muskegon, MI) and the water was purified through a Milli-Q system (Millipore, Bedford, MA). All other chemicals were reagent grade or better and used without further purification.

Chromatographic equipment and conditions

A Dionex PCX-500 HPLC column (polymer reversed-phase/ion-exchange; 46 mm × 250 mm), operating at ambient room temperature, was employed in the analyses, in conjunction with a Dionex PCX-500 HPLC guard column (46 mm × 55 mm). A Beckman 126 Pump was used, operating with a flow rate of 1 ml/min and back-pressure of approx. 2500 p.s.i. A Beckman 507 Autosampler was programmed with an injection volume of 20 μl. A Beckman 168 Diode Array Detector was employed, monitoring at a wavelength of 254 nm, with an absorbance range of 1 V/*A* unit, and a 0.05 s response time. A Beckman System Gold Data Acquisition System was used for integration. The integrator attenuation, peak width, and peak threshold were adjusted for each run. The approximate run time of each analysis was 10 min without a guard column, and 12–15 min with the use of a guard column.

The mobile phase was prepared by mixing appropriate volumes of acetonitrile, 0.6 M perchloric acid solution, and 1.0 M potassium chloride solution with water. A particularly useful mobile phase was prepared by mixing 880 ml of acetonitrile, 75.0 ml of 0.6 M perchloric acid solution, and 450 ml of 1.0 M potassium chloride solution with 595 ml of water. The mobile phase was first filtered (0.45 μm Gelman Acro Disk 50 polyvinylidine difluoride (PVDF)) and subsequently degassed by vacuum sonication.

Analysis of nicotine raw material

Approx. 80 mg of the nicotine free-base sample was accurately weighed into a tared 100 ml volumetric flask, and diluted to volume with water. The solution was subsequently diluted with water to approx. 40 μg/ml. Accurately prepared standard solutions of the nicotine salicylate reference material were typically prepared at 75 μg/ml (equivalent to 40 μg/ml nicotine) in water.

Analysis of transdermal patches

Each patch tested was removed from its release liner, and transferred to a 50 ml volumetric flask so that the final expected concentration of nicotine in the solution would be between 500 and 800 μg/ml. The flask was partially filled with tetrahydrofuran (THF), and the mixture was sonicated for at least 30 min at room temperature; ethanol (absolute) was slowly added to volume. A 5.0 ml aliquot of this solution was transferred to a 100 ml volumetric flask containing 5 ml of dilute perchloric acid (0.6 M). The organic solvents were evaporated by applying a slow nitrogen flow at room temperature. After approx. 45 min under the nitrogen flow, the sample volume decreased by about one-third, and the adhesive-polymer matrix began to flocculate. Water was then added to volume, affording a final nominal concentration of nicotine in the range of 25–40 μg/ml.

Results and Discussion

Effect of mobile phase composition on separations

The chemical structures of known degradation products of nicotine (Thompson et al., 1985; Benowitz and Peyton, 1987; Kyerematen et al., 1987; Sepkovic and Haley, 1987) and related substances are represented in Fig. 1. Authentic samples of nornicotine (Fig. 1b), cotinine (Fig. 1c), nicotine 1-*N*-oxide (Fig. 1d), nicotine 1,1′-di(*N*-oxide) (Fig. 1e), and nicotine 1′-*N*-oxide (Fig. 1f) were obtained and analyzed. The order of elution of these materials under various chromatographic conditions was determined. A typical chromatogram of nicotine salicylate spiked with the known degradation products and related substances is illustrated in Fig. 2. Spiked materials were present in approx. 10% mole ratio compared to nicotine. A typical chromatogram of nicotine salicylate with the known degradation products and related substances present in approx. 1% mole ratio (i.e., more typical of routine samples for analysis) is represented in Fig. 3. The

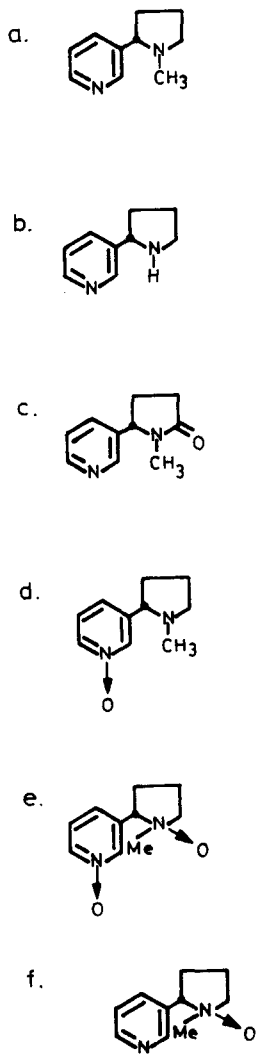

Fig. 1. Structures of related substances and known degradation products of nicotine. (a) Nicotine, (b) nornicotine, (c) cotinine, (d) nicotine 1-*N*-oxide, (e) nicotine 1,1′-di(*N*-oxide), (f) nicotine 1′-*N*-oxide.

excellent peak shapes with a marked absence of significant tailing are of particular note.

Retention of the various analytes was found to be dependent on mobile phase composition. A plot of the log of the capacity factors (k') vs the acetonitrile content in the mobile phase is presented in Fig. 4. A plot of the log of the capacity factors (k') vs the potassium chloride content of the mobile phase is demonstrated in Fig. 5. The retention of salicylic acid and nicotine 1,1′-di(*N*-oxide) decreases with increasing concentration of acetonitrile in the mobile phase, whereas the capacity factors of nicotine, nornicotine, cotinine, and the nicotine *N*-oxides are not significantly affected. Conversely, the retention of nicotine and nornicotine decreases with increasing concentration of potassium chloride in the mobile phase, whereas the capacity factors of salicylic acid and nicotine 1,1′-di(*N*-oxide) are not significantly affected. The retention of cotinine, nicotine 1-*N*-oxide, and nicotine 1′-*N*-oxide also decreases slightly with increasing concentrations of potassium chloride. However, at KCl concentrations greater than 125 mM, these compounds elute very close to the void volume, and a clear change in retention is difficult to observe.

This behavior can be explained based upon the novel characteristics of the new column, the chemistry of the analytes, and the properties of the mobile phase. Salicylic acid and nicotine 1,1′-di(*N*-oxide) are both neutral species at the pH of the eluant (pH 1.2). As expected in the case of uncharged species, these compounds are retained on the column by reversed-phase interactions and not by ion-exchange mechanisms. Consequently, the capacity factors of these compounds are strongly dependent on the acetonitrile content of the mobile phase, and independent of the potassium chloride content.

In contrast, nicotine and nornicotine each carry two positive charges at the pH of the eluant, and thus would be expected to interact with the cation-exchange sites of this column. As such, the capacity factors of these divalent hydrophilic cations are strongly dependent on the concentration of potassium chloride in the mobile phase. Also, since the predominant mechanism of retention is not by reverse-phase interactions, the capacity factors are essentially independent of the acetonitrile content.

The *N*-oxides of nicotine have a net positive charge at the pH of the eluant, and are probably also retained on the column by cation-exchange interactions, as would be expected of monovalent cations. However, the dependence of their capacity factors on the concentration of potassium chloride in the mobile phase (Fig. 5) is obscured

by the close proximity of the elution of these compounds to the void volume.

Cotinine, carrying two positive charges at the pH of the eluant, is also expected to be retained on the column via cation-exchange interactions. Similarly to the case of the *N*-oxides, however, the dependence of the capacity factor on the concentration of potassium chloride in the mobile phase is obscured by the close proximity of cotinine's elution to the void volume. The early elution of this divalent cation is probably due to its more hydrophobic character compared to the other analytes. If a better separation of these early-eluting compounds is desired (for better quantitation of cotinine, for example), their retention may be increased by either decreasing the amount of potassium chloride in the mobile phase, or substituting K^+ with a weaker cation-exchange eluant counterion such as NH_4^+, Na^+, or Li^+.

These experiments constitute an elegant illustration of the combination of reversed-phase and ion exchange modes of the multi-phase polymeric column.

It was determined that the optimal mobile phase composition which adequately separated the compounds of interest was 44% acetonitrile with 56% aqueous, and 225 mM of KCl. Satisfactory resolution was also achieved employing mobile phase compositions in the range 39–49% acetonitrile, and 200–250 mM KCl. Minor varia-

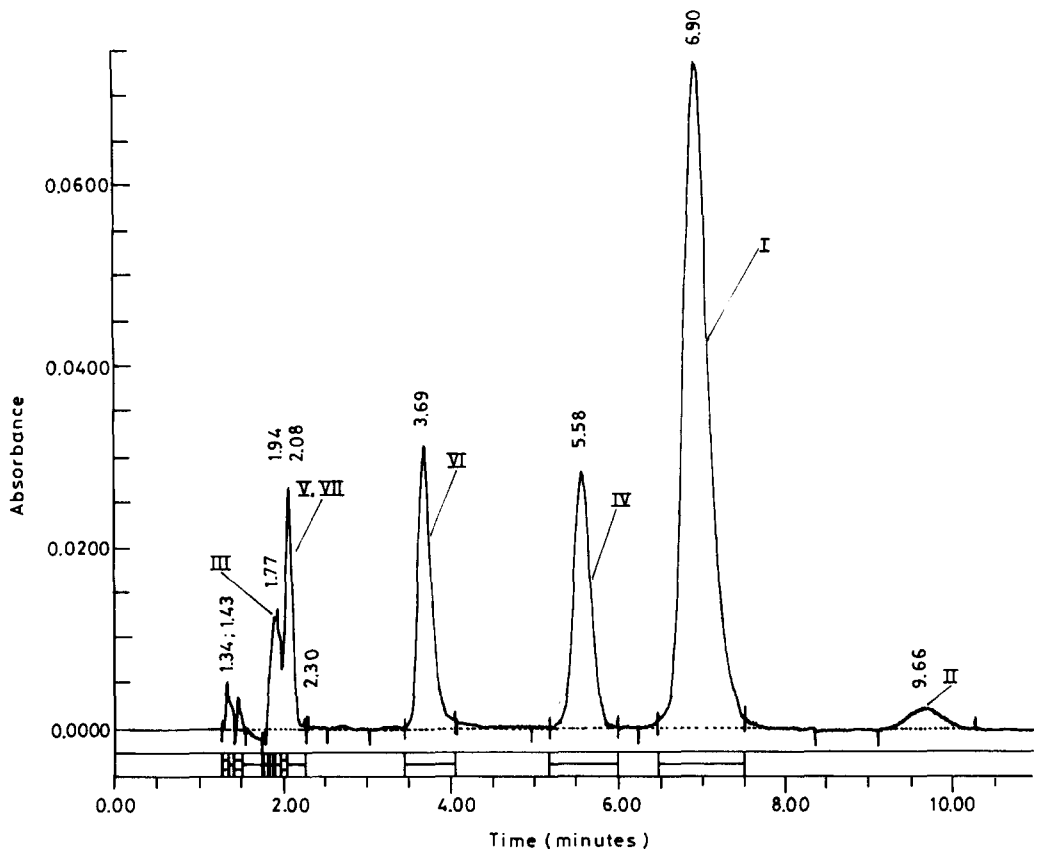

Fig. 2. HPLC chromatogram of nicotine, related substances and known degradation products of nicotine (approx. 10% mole ratio compared to nicotine), and salicylate. Mobile phase: acetonitrile, 0.6 M perchloric acid solution, 1.0 M potassium chloride solution, and water in the proportions 1:6:7.9:11.7, by vol. (I) Nicotine, (II) nornicotine, (III) cotinine, (IV) salicylate, (V) nicotine 1-*N*-oxide, (VI) nicotine 1,1'-di(*N*-oxide), (VII) nicotine 1'-*N*-oxide.

tions in the pH of the mobile phase do not affect the retention of the analytes: at an eluant pH below 2, the analytes are all fully protonated.

The efficiencies of the analytical columns tested were evaluated with respect to nicotine. Columns displayed efficiencies of greater than 2000 theoretical plates over the range of mobile phases tested.

Stability specificity

To confirm the separation capability, and to ensure the integrity of the parent (nicotine) peak, analyses were carried out on active nicotine patches which had been thermally stressed. Three active nicotine patches were stored separately in sealed 20-ml vials each containing one drop of water. The vials were placed in a chamber equilibrated at 60 °C, then removed after 24 days and assayed as above, using the optimum mobile phase composition (under these conditions, approx. 5% degradation occurs). The thermally stressed patches, patch release liners, and vial rinsates were analyzed for nicotine and nicotine degradation products. In a preliminary analysis, the HPLC run time was carried out to 30 min in order to explore the chromatographic region past the nicotine peak for late-eluting compounds; none were detected. A run time of 10 min was then selected for further work. A typical chromatogram is represented in Fig. 6. A nicotine *N*-oxide (or both *N*-oxides coeluting), nicotine 1,1′-di(*N*-oxide), and cotinine were detected,

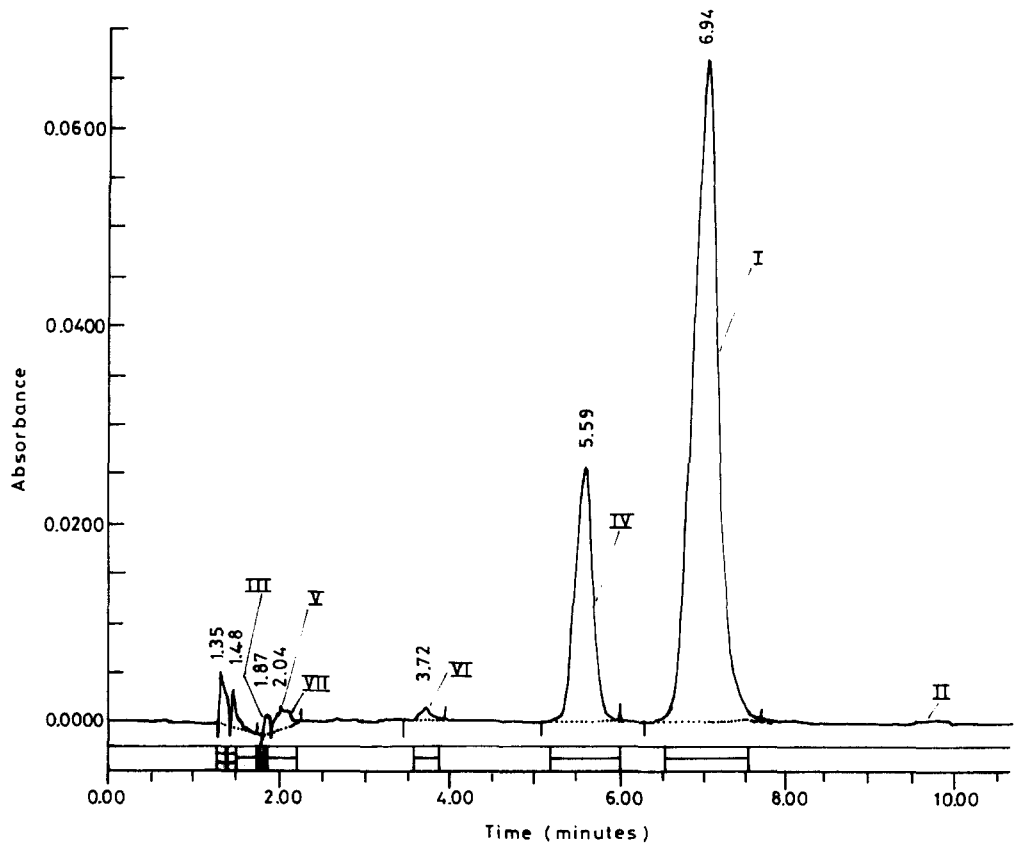

Fig. 3. HPLC chromatogram of nicotine, related substances and known degradation products of nicotine (approx. 1% mole ratio compared to nicotine), and salicylate. Mobile phase: acetonitrile, 0.6 M perchloric acid solution, 1.0 M potassium chloride solution, and water in the proportions 1:6:7.9:11.7, by vol. (I) Nicotine, (II) nornicotine, (III) cotinine, (IV) salicylate, (V) nicotine 1-*N*-oxide, (VI) nicotine 1,1′-di(*N*-oxide), (VII) nicotine 1′-*N*-oxide.

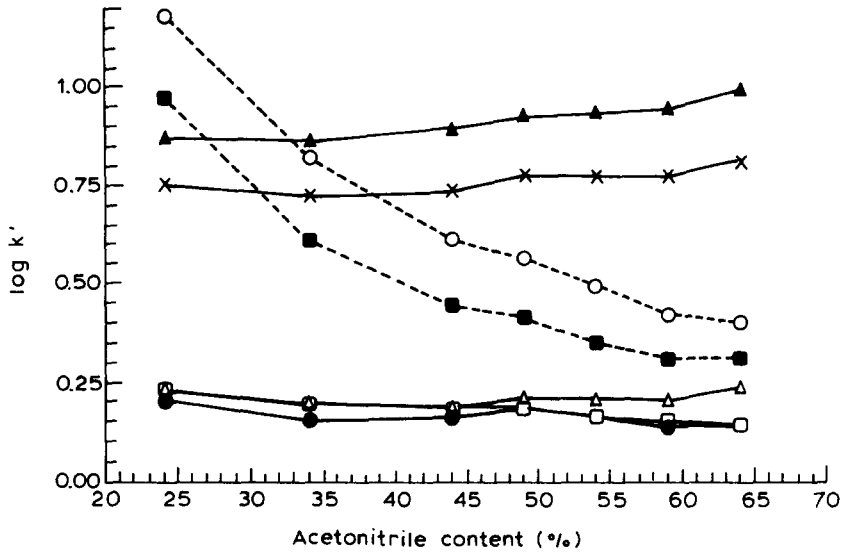

Fig. 4. Log k' for nicotine (×), nornicotine (▲), salicylate (○), nicotine 1-*N*-oxide (■), nicotine 1'-*N*-oxide (△), nicotine 1,1'-di(*N*-oxide) (□), and cotinine (●) as a function of acetonitrile content of the mobile phase. The nominal concentration of potassium chloride was 225 mM.

along with trace amounts of three unidentified compounds. These peaks were not present in the chromatograms of placebo patches stored under identical conditions for the same time period (Fig. 7). It may also be noted that peaks which might interfere with the quantitation of nicotine

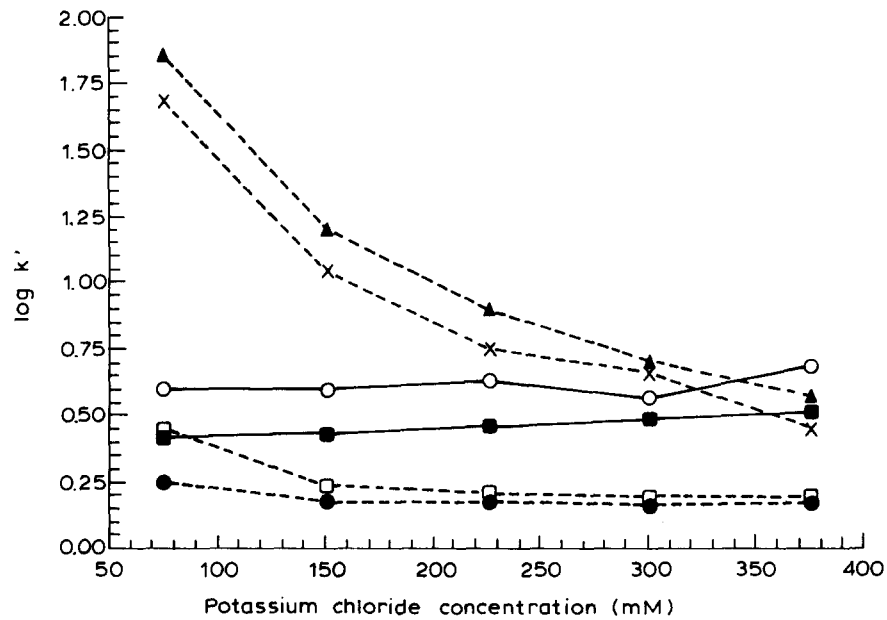

Fig. 5. Log k' for nicotine (×), nornicotine (▲), salicylate (○), nicotine 1-*N*-oxide (■), nicotine 1'-*N*-oxide co-eluting with nicotine 1,1'-di(*N*-oxide) (□), and cotinine (●) as a function of potassium chloride content of the mobile phase. Acetonitrile content was held constant at 44%.

were absent in the chromatogram of placebo patches which had been thermally stressed.

The spectral homogeneity of the nicotine peak in the stressed active patch samples was evaluated employing a diode array spectrophotometer. A spectral overlay plot for three segments of the nicotine band of the chromatogram of the thermally stressed patch sample is presented in Fig. 8. UV spectra of the upslope (9.72 to 9.82 min), apex (10.01 to 10.11 min), and downslope (10.45 to 10.55 min) portions of the sample band were obtained. The three spectra were normalized with respect to the apex spectrum, and overlaid. Excellent agreement was obtained between the three spectra: correlation coefficients for the upslope/apex, apex/downslope, and upslope/downslope overlays were 0.9682, 0.9933, and 0.9384, respectively.

First-derivative curves of the UV spectra of the same upslope, apex, and downslope portions of the sample band were obtained (Fig. 9), highlighting the inflection points of the corresponding absorbance spectra. The three curves were normalized with respect to the apex spectrum, and overlaid. Excellent agreement was obtained between the three curves: correlation coefficients for the upslope/apex, apex/downslope, and upslope/downslope overlays were 0.9971, 0.9947, and 0.9852, respectively.

UV absorbance ratio plots were obtained for

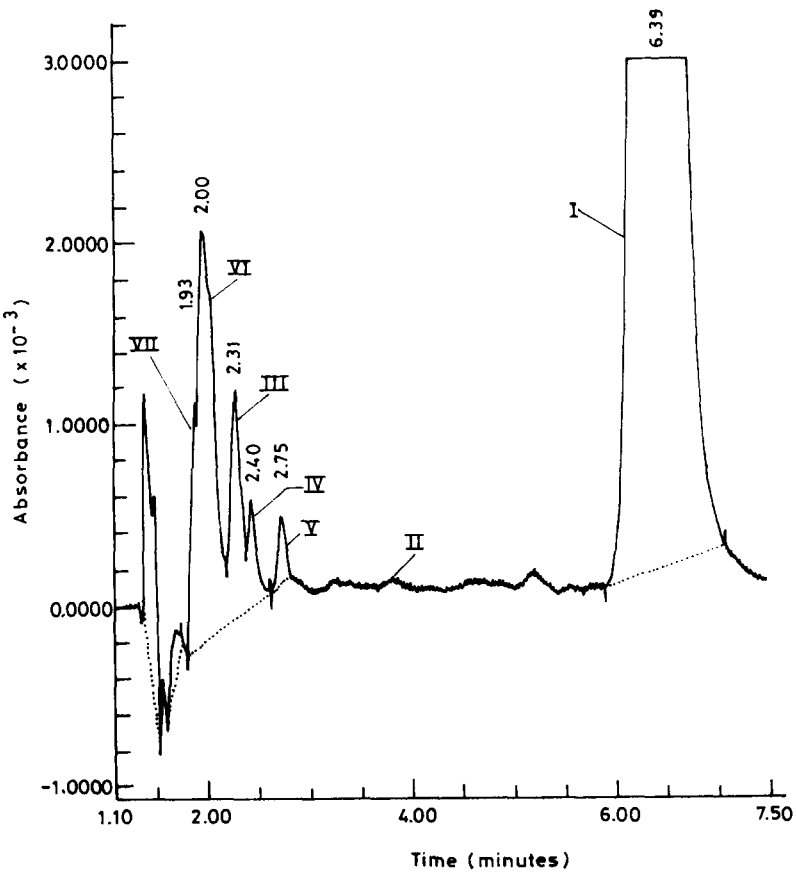

Fig. 6. HPLC chromatogram of rinsate of vial which had contained thermally stressed nicotine patch (stress conditions = 60 °C for 24 days). Mobile phase: acetonitrile, 0.6 M perchloric acid solution, 1.0 M potassium chloride solution, and water in the proportions 1:6:7.9:11.7, by vol. (I) Nicotine, (II) nicotine 1,1'-di(*N*-oxide), (III) unknown, (IV) unknown, (V) unknown, (VI) nicotine 1-*N*-oxide and/or nicotine 1'-*N*-oxide, (VII) cotinine.

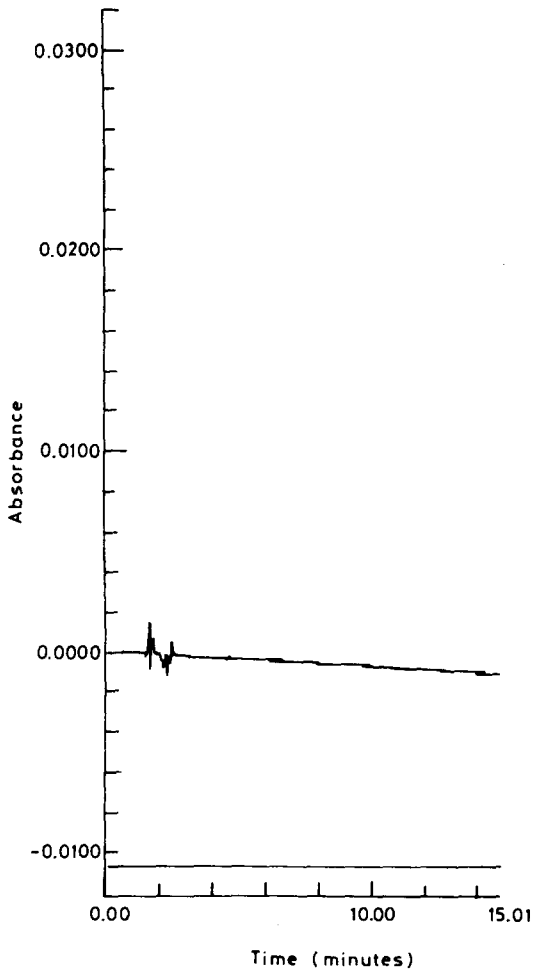

Fig. 7. HPLC chromatogram of placebo patch sample which had been stored for 24 days at 60 °C. Mobile phase: acetonitrile, 0.6 M perchloric acid solution, 1.0 M potassium chloride solution, and water in the proportions 1:6:7.9:11.7, by vol.

the upslope (9.72 to 9.82 min), apex (10.01 to 10.11 min), and downslope (10.45 to 10.55 min) segments of the sample band, monitoring at 254 and 264 nm. The UV absorbance ratio plot for the upslope segment of the nicotine band of a thermally stressed nicotine transdermal patch sample is represented in Fig. 10. For the upslope sample band, the ratio of absorbance monitored at 254 nm to that at 264 nm was 1.037. Absorbance ratios of 1.041 and 1.050 were obtained for the apex and downslope sample bands, respectively. The coefficient of variation between the three values was calculated to be 0.6%, indicating peak homogeneity throughout the region of interest.

The chromatographic conditions employed in this method permit satisfactory resolution of the compounds of interest. It was concluded that the method is specific with regard to the known ordinary impurities and degradation products of nicotine at the monitoring wavelength (254 nm).

Method accuracy and linearity

The accuracy and linearity of the method were evaluated by analysis of placebo patch test samples to which known amounts of nicotine had been added. Ten concentration levels were evaluated, ranging from 0.434 to 62.0 μg/ml in the assay solution. A placebo stock solution was first prepared by dissolving two placebo patches in 50 ml of THF followed by dilution of the preparation to 100 ml with ethanol (absolute). A nicotine stock solution was prepared by weighing 62.0 mg of nicotine free-base into a 100 ml volumetric flask, which contained approx. 20 ml of water and 5 ml of 0.6 M $HClO_4$. The solution was thoroughly mixed and brought to volume with water, affording a final nicotine concentration of 620 μg/ml.

Spiked placebo solutions of various concentrations were obtained by the addition of aliquots of the nicotine stock solution to 5.0 ml of placebo stock solution in a 100 ml volumetric flask containing 5 ml of 0.6 M $HClO_4$. The organic solvents were evaporated by applying a slow nitrogen flow at room temperature. After approx. 45 min under the nitrogen flow, the sample volume decreased by about one-third, and the adhesive-polymer matrix began to flocculate. Water was then added to volume. Quantitative transfer of 10.0-, 9.0-, 7.0-, 5.0-, 3.0-, and 2.0-ml aliquots of the nicotine stock solution afforded spiked placebo solutions with expected concentrations of 62.0, 55.8, 43.4, 31.0, 18.6, and 12.4 μg/ml, respectively. Subsequent dilution of 10 ml of the 43.4-, 31.0-, and 18.6-μg/ml solutions to 100 ml afforded spiked placebo solutions of expected concentrations of 4.34, 3.10, and 1.86 μg/ml, respectively. Subsequent dilution of 10 ml of the first of these solutions to 100 ml afforded a

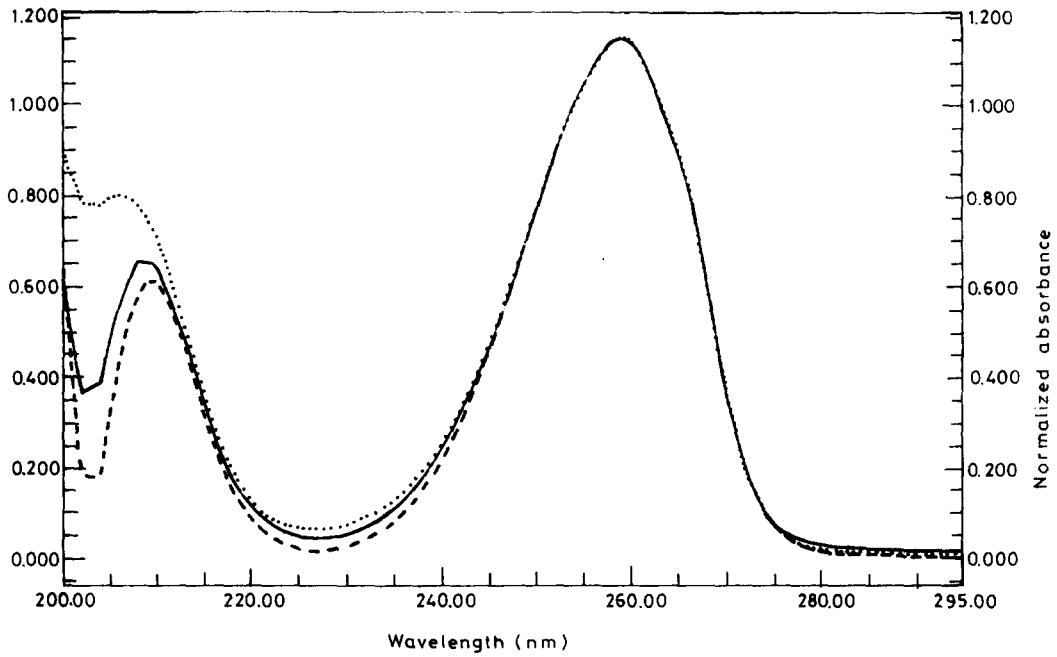

Fig. 8. Spectral overlay plot for the upslope, apex, and downslope of the nicotine band in a thermally stressed transdermal patch sample (stress conditions = 24 days at 60 °C). (———) Upslope, (· · · · · ·) apex, (- - - - -) downslope.

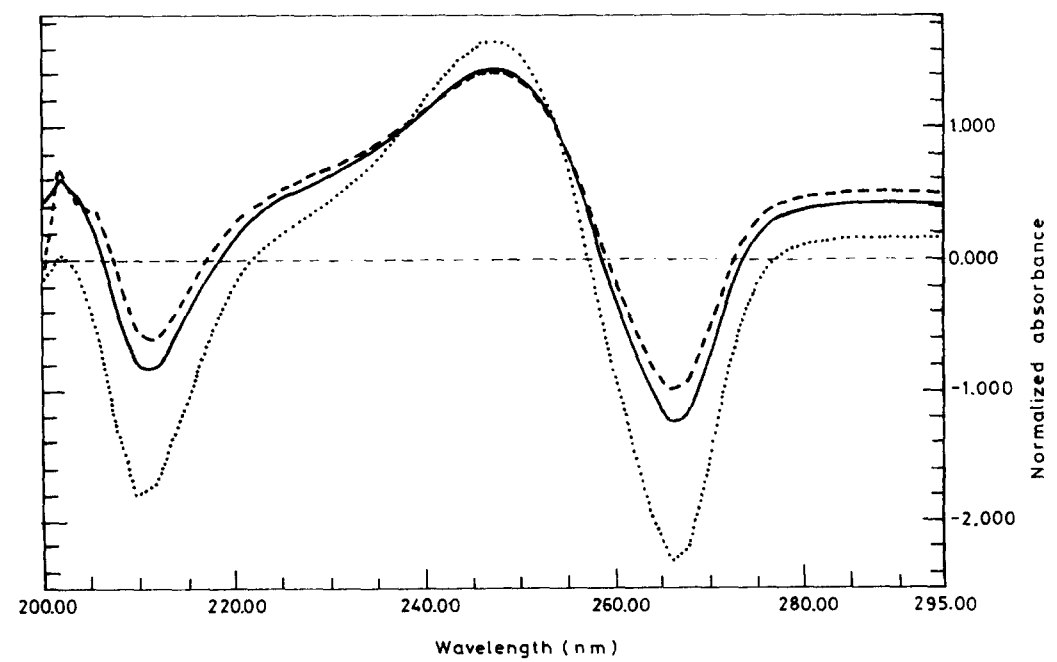

Fig. 9. Normalized first derivative of spectral overlay plot for the upslope, apex, and downslope of the nicotine band in a thermally stressed transdermal patch sample (stress conditions = 24 days at 60 °C). (———) Upslope, (· · · · · ·) apex, (- - - - -) downslope.

TABLE 1

Accuracy as the amount of nicotine found in spiked placebo samples compared to amounts added

Expected concentration (μg/ml)	Determined concentration (μg/ml)			
	Peak area	% recovery	Peak height	% recovery
0.434	0.435	100	0.444	102
1.86	1.83	98.5	1.83	98.6
3.10	3.05	98.3	3.12	101
4.34	4.29	98.9	4.33	99.8
12.4	12.4	100	12.5	101
18.6	18.6	99.9	19.0	102
31.0	30.8	99.3	31.1	100
43.4	43.4	100	43.2	99.6
55.8	55.8	100	54.5	97.6
62.0	62.0	100	60.2	97.0

spiked placebo solution containing 0.434 μg/ml of nicotine.

Each sample was analyzed in duplicate. Quantitation was carried out based on peak area. Quantitation was also accomplished based on peak heights, for comparison purposes. The results of this experiment, calculated on the basis of peak height, are presented in Table 1.

At approx. 100% of the nominal sample concentration (25–40 μg/ml), the percent recovery was found to be 99.7% when integration was performed using peak area, and 100.5% when quantitation was carried out according to peak height. The difference between the known concentrations of the added materials and the concentrations determined by the method was found to be within experimental error. Linear least-squares regression curves were prepared for the values determined via HPLC analysis vs the expected nicotine concentration values. When integration was performed by peak area, the correlation coefficient of the regression line was calculated to be 1.0000. The slope was determined to be 1.0001 ± 0.0001, and the y-intercept was evaluated to be -0.03 ± 0.03. When quantitation was performed on the basis of peak height, the corre-

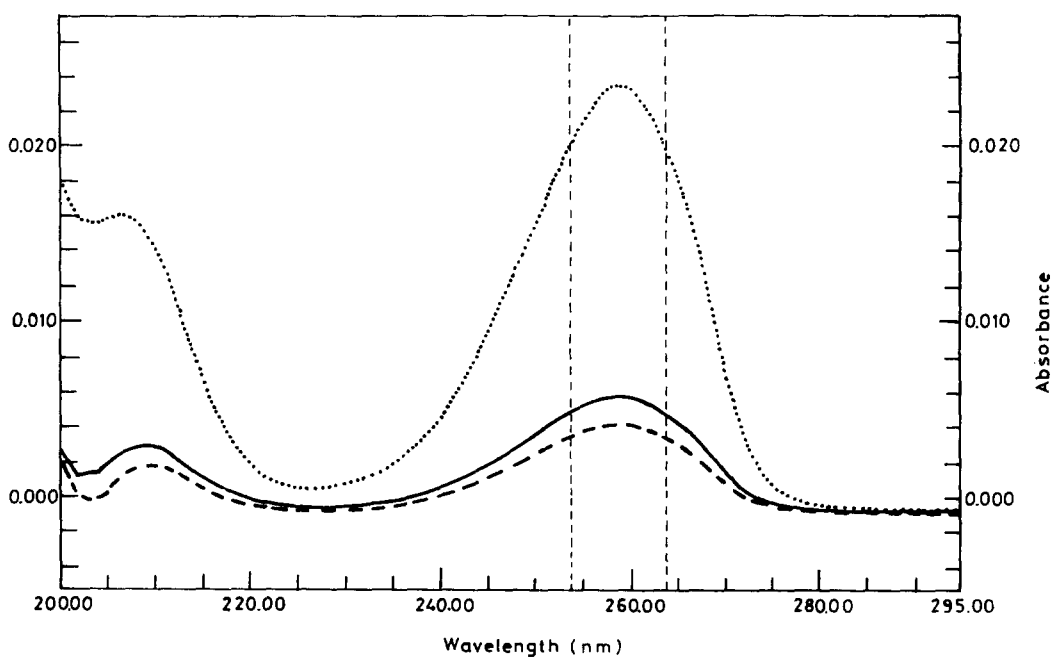

Fig. 10. UV absorbance ratio plot for the upslope (9.72 to 9.82 min) segment of the nicotine band of a thermally stressed transdermal patch sample (stress conditions = 24 days at 60 °C). (———) Upslope, (· · · · · ·) apex, (- - - - -) downslope.

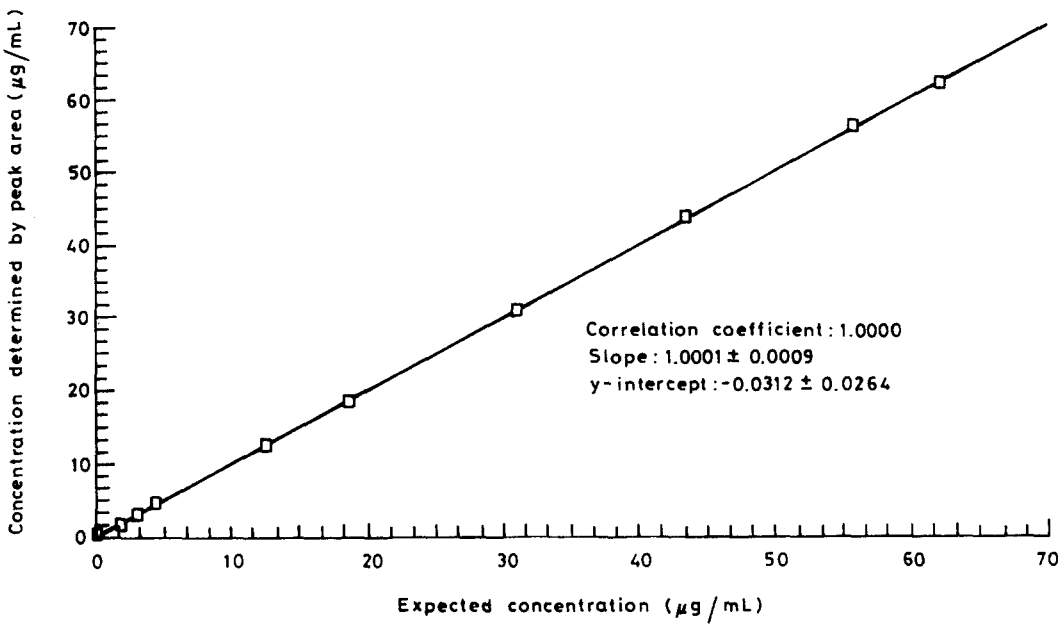

Fig. 11. Linear relationship between nicotine concentration and peak response for concentrations between 0.434 and 62.0 μg/ml. Nicotine concentrations were determined based on a single reference standard solution (43.4 μg/ml); quantitation by peak area.

lation coefficient of the regression line was calculated to be 0.9997. The slope was determined to be 0.977 ± 0.006, and the y-intercept was evaluated to be 0.2 ± 0.2. The chromatography shows a linear relationship between nicotine concentration and peak response determined on the basis

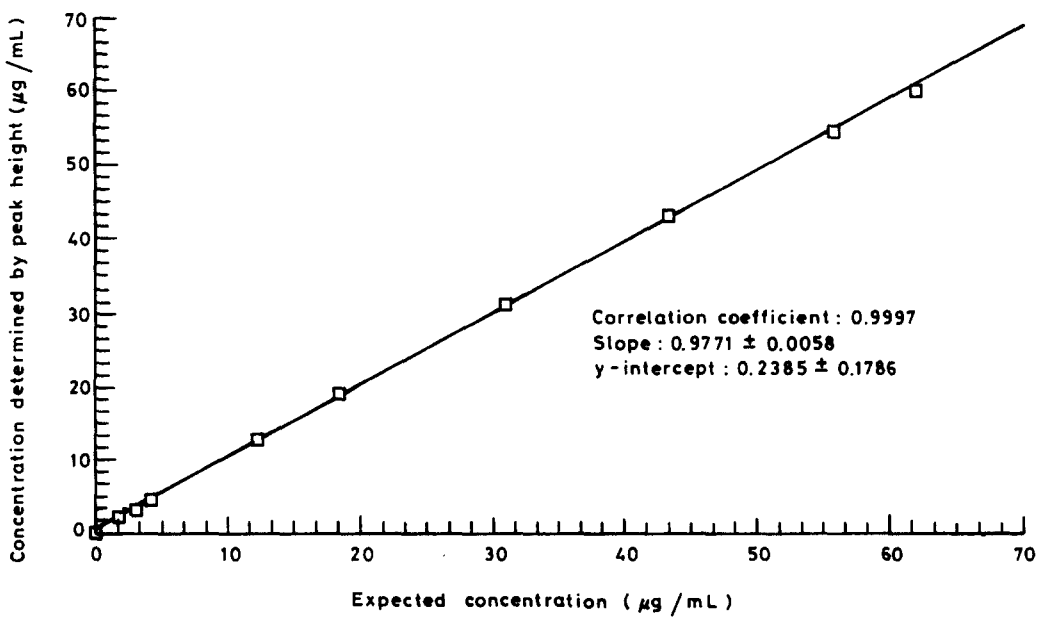

Fig. 12. Linear relationship between nicotine concentration and peak response for concentrations between 0.434 and 62.0 μg/ml. Nicotine concentrations were determined based on a single reference standard solution (43.4 μg/ml); quantitation by peak height.

of either peak area or peak height for concentrations between 0.434 and 62.0 μg/ml (Figs 11 and 12, respectively).

In another experiment, the effect on accuracy and precision of a filtration step during sample preparation was investigated. Ten individual nicotine patches were assayed without prior filtration of the sample matrix. Three sets of samples were filtered through Uniflow cellulose acetate (0.45 μm) filters, Waters Millex polyvinylidine difluoride (0.45 μm) filters, and Anatop inorganic fiberglass filters (0.2 μm), respectively, prior to HPLC analysis.

The results of the replicate determinations are listed in Table 2. Although method precision was adequate in all cases, filtration of the samples prior to HPLC analysis appears to introduce an unacceptable bias to the results. Filtration of assay samples is therefore proscribed.

The method was thus found to be accurate and linear with respect to the quantitation of nicotine. Quantitation by peak area is recommended; quantitation by peak height also gives suitable accuracy and linearity.

Chromatography precision

The chromatography precision was evaluated by assessment of the degree of variation between replicate injections of nicotine transdermal patch sample solutions. One nicotine transdermal patch was prepared for assay, and injected six times. Three sets of samples were filtered through Uniflow cellulose acetate (0.45 μm) filters, Waters Millex polyvinylidine difluoride (0.45 μm) filters, and Anatop inorganic fiberglass filters (0.2 μm), respectively, prior to HPLC analysis. Each of these sample preparations was injected six times; integration was performed by peak area. The results of the analysis are presented in Table 3. The observed coefficient of variation was determined to be 0.2% for unfiltered samples, and 0.5, 0.2, and 0.5% for samples filtered employing cellulose acetate, polyvinylidine difluoride, and fiberglass filters, respectively. The precision of the chromatography was thus evaluated and demonstrated to be adequate.

Method precision

Precision of the method was estimated by assessment of the degree of variation between replicate analyses of nicotine transdermal patches (note that this estimation is biased on the high side, since as carried out, the estimate includes a contribution from patch-to-patch variability as well as intrinsic method variability). Ten individual nicotine patches were assayed without prior

TABLE 2

Method precision, as replicate analyses of nicotine transdermal patches

Sample no.	Determined drug content (mg/patch)			
	Unfiltered	Filtered: CA [a]	Filtered: PVDF [b]	Filtered: inorganic [c]
1	38.2	38.2	40.5	35.6
2	37.7	37.6	39.2	35.9
3	38.3	38.0	39.6	36.7
4	37.1	36.2	37.3	35.6
5	38.9	37.8	40.2	35.7
6	37.2	36.8	38.1	34.1
7	37.1	36.9	39.5	35.0
8	37.3	37.1	38.3	34.8
9	37.1	37.3	39.6	34.8
10	38.3	37.9	39.6	35.3
Average:	37.7	37.4	39.2	35.4
Standard deviation:	0.6	0.6	1.0	0.7
Coefficient of variation (%)	1.7	1.7	2.5	2.0

[a] Uniflow cellulose acetate (0.45 μm).
[b] Waters Millex polyvinylidine difluoride (0.45 μm).
[c] Anatop Plus inorganic fiberglass (0.2 μm).

filtration of the sample matrix. The results of the replicate determinations are presented in Table 2. The observed coefficient of variation among individual test results was determined to be 1.7% for unfiltered samples, and it was concluded that method precision was adequate in all cases. The degree of reproducibility of the analytical method under normal operating conditions was thus established and the precision of the method was determined. The results of the filtration variants in Table 2 also demonstrate the excellent precision of the method (despite the bias in accuracy introduced by the filtration step).

Limits of quantitation and detection

The limit of quantitation of nicotine under the HPLC conditions delineated in this report was determined to be 0.4 μg/ml, and the limit of detection was evaluated to be 0.2 μg/ml. Limits of detection of the related substances and known degradation products of nicotine were estimated to be approx. 0.4 μg/ml, based on the expectation that compounds of similar structure will have similar UV absorptivities.

Storage and reassay of assay sample sets

On occasion, it is desirable to re-run chromatography sample sets (if, for instance, an instrument malfunction occurs). The applicability of this procedure was evaluated since nicotine may slowly evaporate from aqueous solutions, possibly introducing an error. Different rates of evaporation of nicotine from standard and sample solutions would be expected since the sample solutions contain perchloric acid, which would be expected to retard the evaporation process.

TABLE 4

Stability of sample preparations: initial results and results after storage for three days at ambient room temperature

Sample no.	Drug content (mg/patch)	
	Initial	Day 3
1	38.2	38.4
2	37.7	37.8
3	38.3	38.7
4	37.1	37.1
5	38.9	38.7
6	37.2	37.8
7	37.1	37.5
8	37.3	37.8
9	37.1	37.9
10	38.3	37.8
Average:	37.7	38.0
Standard deviation:	0.6	0.5
Coefficient of variation (%)	1.7	1.3

TABLE 3

Chromatography precision, as analyzed by six replicate injections of both filtered and unfiltered samples

Injection no.	Detector response (peak area) (A s)			
	Unfiltered	Filtered:		
		Cellulose acetate [a]	PVDF [b]	Fiberglass [c]
1	17.06	17.16	18.22	15.87
2	17.07	17.13	18.25	15.94
3	17.04	17.11	18.21	15.81
4	16.97	17.05	18.18	15.78
5	17.03	17.03	18.23	15.78
6	17.04	16.93	18.25	15.74
Average:	17.03	17.07	18.22	15.82
Standard deviation:	0.04	0.09	0.03	0.08
Coefficient of variation (%)	0.2	0.5	0.2	0.5
Nicotine concentration in injectate (μg/ml):	37.2	37.3	39.8	34.6

[a] Uniflow cellulose acetate (0.45 μm).
[b] Waters Millex polyvinylidine difluoride (0.45 μm).
[c] Anatop Plus inorganic fiberglass (0.2 μm).

Two working reference standard solutions (37.1 μg/ml) and 10 sample preparations were analyzed, stored at ambient room temperature (approx. 25 °C) for 3 days, and subsequently re-assayed. The results of the analysis of these samples, both initially and after storage for 3 days, are presented in Table 4. The average difference was 0.8% or less for each set of samples. The results indicate that chromatography sample sets (assay samples plus reference solutions) may be stored and re-analyzed if necessary for up to at least 3 days.

Conclusions

This report describes a rapid, precise, stability-indicating method to analyze nicotine as a raw material or in transdermal dosage forms using HPLC with UV detection. The method uses a new multi-phase polymeric column which allows separation by a combination of reversed-phase and ion-exchange chromatography. The optimal mobile phase composition and an acceptable composition range were determined by evaluation of the change in capacity factor values of nicotine, nicotine 1-*N*-oxide, nicotine 1′-*N*-oxide, nicotine 1,1′-di(*N*-oxide), nornicotine, and cotinine as a function of mobile phase composition. The method was further characterized with respect to specificity, linearity, accuracy, and precision. It was demonstrated that the chromatographic conditions employed in this method permit satisfactory resolution of the compounds of interest: the method is specific with regard to the known ordinary impurities and degradation products of nicotine at the monitoring wavelength (254 nm). The method was found to be accurate and linear with respect to the quantitation of nicotine, even after storage of the assay samples for 3 days. The precision of the chromatography was also evaluated and demonstrated to be adequate.

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