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Development and validation of a gradient reversed-phase high-performance liquid chromatographic assay for *S*(−)-2-(N-propyl-N-2-thienylethylamino)-5-hydroxytetralin (N-0923) from a transdermal delivery system

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Abstract

A gradient reversed-phase HPLC method for potency determination of N-0923 (10 mg) from a transdermal delivery system (TDS), was developed and validated with single point calibration using internal standard quantitation. N-0923 and the internal standard, N-0434, are eluted from a reversed-phase C_{18} column using a gradient which contains 0.1 *M* triethylamine–0.04 *M* citrate buffer, pH 5.9, water, and acetonitrile with UV detection at 272 nm. N-0923 is isolated from the transdermal delivery system by extraction with *n*-heptane followed by extraction of the resulting organic phase with 0.1 *M* citric acid containing the internal standard. The method was free from matrix interferences in both untreated and forced degraded placebo delivery systems. Acceptable linearity and quantitative recovery from spiked placebo delivery systems over the range 50–150% of nominal label claim were demonstrated. Within-day assay precision from individual samples of active transdermal delivery systems ($n = 10$) was 5.6% R.S.D. The detection limit was at least 0.1 μg/ml which is equivalent to 0.05% of the working standard concentration. Replicate injection precision at this level was 0.08% R.S.D. ($n = 4$). Analysis of thermally stressed active and placebo delivery systems with this HPLC method and photodiode-array detection showed that the chromatography was stability-indicating as demonstrated by the absence of measurable interferences from principal degradation products of either the N-0923 or the delivery system excipients.

1. Introduction

N-0923 [*S*(−)-2-(N-propyl-N-2-thienylethylamino)-5-hydroxytetralin HCl] is a potent, selective D_2 dopamine receptor agonist (Fig. 1) [1–3]. The antiparkinson activity of this aminotetralin was first identified in a study using the racemate, N-0437, in 1-methyl-4-phenyl-1,2,3,6-tetrahydro-

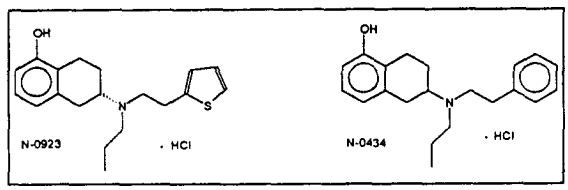

Fig. 1. Chemical structures of N-0923 and N-0434 (internal standard).

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pyridine (MPTP)-lesioned marmosets [4]. Studies of the (−)enantiomer, N-0923, identified it as the active enantiomer using the 6-hydroxy dopamine rat and the MPTP monkey models [5–7]. The large first pass metabolism and subsequent rapid clearance make oral dosing of N-0923 impractical. The feasibility of transdermal delivery has been evaluated using a novel transdermal patch developed by Cygnus Therapeutic Systems in conjunction with Whitby Research.

Various HPLC methods have been reported for measuring N-0923 in animal or human plasma [8–12]. A few analytical methods for analysis of pharmaceuticals from transdermal formulations have been published [13–16]. A facile and rugged analytical method was needed for release and stability testing of the N-0923 10 mg transdermal delivery system (TDS). This reversed-phase method was designed to elute the N-0923 (I) peak under isocratic conditions, followed by gradient elution of previously observed synthetic impurities and degradation products. Because of the need for solvent extraction to release the drug from the delivery system, an internal standard, N-0434 (II) (Fig. 1), was used to improve both the accuracy and the precision of the method.

2. Experimental

2.1. Materials

HPLC grade acetonitrile (ACN) (Burdick and Jackson, Muskegon, MI, USA) was purchased from Baxter (Columbia, MD, USA). Water was prepared by reverse osmosis and deionization using a MilliQ system (Millipore, Milford, MA, USA). Citric acid (99.5 + % anhydrous) was purchased from Aldrich (Milwaukee, WI, USA). Triethylamine (TEA) (analyzed reagent grade) was obtained from J.T. Baker (Phillipsburg, NJ, USA) and *n*-heptane, HPLC grade, was obtained from Fisher (Pittsburgh, PA, USA). N-0923 (I) and N-0434 (II) (internal standard) were obtained from Whitby Research (Richmond, VA, USA). I Transdermal delivery systems (lot 27242) and placebo transdermal delivery systems (lot 27214A) were manufactured by Cygnus Therapeutic Systems (Redwood City, CA, USA). Filtration of working samples was carried out using Gelman Nylon Acrodisc 13 mm 0.5 μm pore size filters (Baxter, Columbia, MD, USA).

2.2. Chromatographic system for assay

The chromatographic system included a SpectraPhysics pump Model P2000, with gradient capabilities, a SpectraPhysics Solvent degaser, Model SCM400 (Fremont, CA, USA), a Waters 490E programmable multi-wavelength UV detector and a Waters 717 autosampler (Milford, MA, USA). The chromatograms were collected using the Waters 860 Expert Ease 3.1 computer software. The analytical column used was a Jones (Lakewood, CO, USA) Apex C_{18}, 5 μm (4.6 mm × 15 cm) in conjunction with a RP-18, 7 μ, 3.2 mm × 15 mm guard column from Applied Biosystems (Foster City, CA, USA). Three separate solvent reservoirs containing 0.1 *M* TEA/0.04 *M* citrate, pH 5.9, water, and ACN were used. The solvent mixture and gradient control was maintained by the SpectraPhysics pump. The mobile phase was isocratic for the first 6 min with 0.1 *M* TEA/0.04 *M* citrate, pH 5.9–water–ACN (10:38:52). After elution of I and II, the gradient was initiated and the mobile phase composition was then increased linearly over 3 min to 0.1 *M* TEA/0.04 *M* citrate, pH 5.9–water–ACN (10:4:86), and maintained there for 18 min to elute a highly non-polar I impurity which had been identified during raw material testing and evaluation. From 18 to 21 min the mobile-phase composition was linearly ramped back to the initial conditions and then re-equilibrated for 14 min. The flow-rate was 1.5 ml/min which produced a maximum column backpressure of 1200 p.s.i. (1 p.s.i. = $6.9 \cdot 10^3$ Pa). The injection volume was 40 μl and the total run time was 35 min. The detection wavelength was 272 nm. The structure of the late eluting I impurity has not yet been determined.

2.3. Chromatographic system for photodiode-array analysis

Thermally stressed TDS samples were prepared by heating placebo and active patches at

80°C for 7 days. Samples were assayed after 25, 72 and 168 h storage under these conditions. Analysis of thermally stressed samples was performed using a SpectraPhysics system ISM 100 with a Waters 990 photodiode-array detector (PDA). Data was collected over a wavelength range of 210 to 399 nm. All other equipment and HPLC conditions were the same as described above.

2.4. Standard preparation

Internal standard solution (II, 400 μg/ml) was prepared in 0.1 *M* citric acid. Stock calibration standard (I, 1 mg/ml) was prepared in 0.1 *M* citric acid–ACN (50:50). Stock solutions were shown to be stable for at least 2 weeks under refrigeration (5–10°C). A working standard was prepared from the stocks containing 200 μg/ml of both I and II diluted to volume with ACN (equivalent to 100% nominal label claim for a 10-mg patch). A verification standard, used to confirm the accuracy of the stocks solution, was prepared in the same manner as the working standard solution.

2.5. Sample preparation

The TDS samples were extracted with 10 ml of *n*-heptane. The mixture was then extracted with 25 ml of internal standard solution. The samples were then centrifuged for 10 min at 2500 *g*. A 5.0-ml aliquot of the aqueous layer was transferred to a 10-ml volumetric flask and diluted to volume with ACN. A portion of the resulting solution was then filtered through an Acrodisc into a polypropylene autosampler vial for analysis.

2.6. Method validation

System suitability parameters were based on the validation results. Instrument precision was demonstrated by four replicate injections of the working standard with R.S.D. ≤1.0% by peak-area ratio. Resolution between I and II was ≥1.5. The USP tailing factor at 5% was ≤1.5 and the plate count, calculated by the USP tangent method was ≥2000. The difference between the average response factor for the working standard and the response factor for the verification standard was ≤2.0%, where the response factor = peak area ratio/concentration. System suitability was determined at the beginning of each run.

Samples were analyzed using single point calibration. One working standard was run with every three samples to ensure the accuracy of the assay over time. Unknowns were quantified using the calibrator injected just prior to the samples. The run was considered acceptable if the mean response factor (response/concentration) for the four replicate injections of the working standard and the response factor for the verification standard differed by less than 2%. The retention times for II and I were typically 4.7 and 5.6 min, respectively.

A 10 mg/ml stock solution, prepared in methanol, was used as a spiking solution to prepare both recovery samples and a calibration standard. Recovery samples were each prepared in duplicate. Placebo TDS samples were spiked in duplicate at five levels to produce concentrations equivalent to 50, 75, 100, 125, and 150% of nominal label claim (10 mg). The methanol was evaporated under nitrogen, and the systems were extracted as described under sample preparation. The calibration standard was prepared as described above with the absence of a TDS. Method precision was assessed by assaying 10 active transdermal delivery systems. The selectivity of the method was established by analysis of unstressed and force-degraded active and placebo TDS samples. Duplicates of force-degraded active (lot 27242A) and placebo (lot 27214A) TDS samples were assayed using PDA detection following 25 h, 72 h, and 7 days of thermal exposure.

3. Results and discussion

3.1. Internal standard selection

An internal standard (I.S.) was added to this method to improve erratic recovery observed during method development. Because of the large percentage of organic solvent in the working samples and the long run times (35 min/

sample), evaporation during a run was also a concern. The internal standard would also correct for the latter situation, if present. Compound II was chosen for its structural similarity to I and because II could be detected at the same UV absorption wavelength. The two peaks, I and II, were well resolved in this chromatographic system ($R > 1.5$) (Fig. 2).

3.2. Sample preparation

Two methods of sample preparation were evaluated. The initial sample preparation procedure suggested by Cygnus involved extraction of I from the TDS into *n*-heptane, followed by addition of methanol. A 5:10 dilution of the methanolic sample solution was made with water in order to precipitate the polymer.

Several potential problems were observed with this method. Addition of methanol resulted in lumpy deposits on the surface of the backing and the surface of the jar. A small heptane layer could also be observed on the top of the methanol–water solution in the neck of the flasks. Injections of both layers indicated partitioning of I and II between the two layers. Partitioning of I was greater (13–20% in *n*-heptane) than II. In addition, *n*-heptane was partitioned into the methanolic layer. The heptane peak did not interfere with I or II, but was highly variable from sample to sample, which would have complicated accurate determination of degradation products during TDS stability testing.

The effect of pH was evaluated by extracting I from *n*-heptane using equal volumes of water, 0.1 *M* TEA/0.04 *M* citric acid pH 5.9 buffer (mobile phase buffer), 0.1 *M* citric acid or 0.1 *M* phosphoric acid. The heptane layer in each extraction was collected and evaporated under nitrogen, reconstituted in mobile phase and injected. The results showed that I partitioned into heptane in both aqueous and pH 5.9 buffered conditions (Table 1). The partitioning of I into heptane with TEA/citric acid buffer was highly significant (mean 29 and 41% for II and I, respectively).

The higher partitioning in the presence of the mobile phase buffer may have resulted from partitioning of the TEA into the heptane layer. Extraction using either 0.1 *M* citric acid or 0.1 *M* phosphoric acid resulted in no partitioning (Table 1); therefore, citric acid was used as the solvent for the internal standard and to extract I from the *n*-heptane. No partitioning of the *n*-heptane into citric acid was observed.

3.3. Specificity

A chromatogram of extracted product placebo at three times the nominal sample size and a chromatogram of forced degraded (80°C for 7 days) product placebo are shown in Fig. 3. No significant peaks are seen in the regions where II

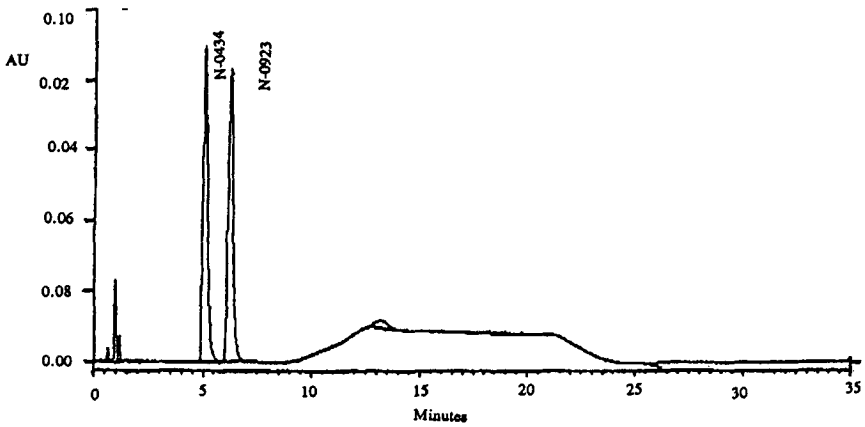

Fig. 2. Typical chromatogram of N-0923 and N-0434.

Table 1
Partitioning of N-0434 and N-0923 into water, mobile phase buffer, 0.1 *M* citric acid and 0.1 *M* phosphoric acid

Sample replicates	Solvent	N-0434 partitioned into heptane (%)	N-0923 partitioned into heptane (%)
1	Water	1.498	2.546
2	Water	2.610	4.441
3	Water	1.544	2.664
1	Buffer	29.341	41.296
2	Buffer	31.086	43.721
3	Buffer	28.057	39.436
1	Citric	0	0
2	Citric	0	0.003
3	Citric	0	0
1	Phosphoric	0	0
2	Phosphoric	0	0.007
3	Phosphoric	0	0

and I elute in either chromatogram, indicating specificity of the method against matrix interferences. Peak purity was determined with a photodiode-array detector for untreated and thermally degraded active TDS samples. The spectral peak purity and potency differences after 7 days between treated and untreated samples were 0.44% and 0.76%, respectively. This is well within the normal inter-sample variability of the methodology (5.6%).

When compared with I raw material, additional peaks at 0.9 and 1.1 min were observed in all unstressed placebo and active TDS samples (Fig. 4), and the 1.1-min peak was also observed in a citric acid blank, indicating that these peaks correspond to placebo components and/or citric acid but not to degradation products of I. This was further confirmed by the lack of change in either of these peaks during the 7-day thermal stressing period. The HPLC conditions for this method have also been used for analysis of I raw material and an aqueous parenteral formulation. Specificity of the chromatography against I degradation products was shown previously [17].

3.4. Linearity and recovery

Single point calibration was chosen for analysis because of its simplicity and wide use in the pharmaceutical industry for content uniformity analysis. The primary assumptions of single point analysis are that the response is linear through the range of interest and that there is no bias in the intercept. If either of these assumptions are violated, the resulting analysis may be inaccurate. Differences in slope may not have a significant effect on values close to the calibration point, but higher or lower concentrations may be substantially under- or over-estimated. A highly positive or negative intercept would suggest consistent under- or over-estimation of measured concentrations (especially those closer to the intercept). Although the method was validated over a range of 50–150% of nominal label claim, the release requirement for the dosage form was ±10% of nominal label claim. The spiked placebo recovery data (Table 2) over the range 50–150% of label claim using a single calibration level at 100% label claim are plotted in Fig. 5. The data were fitted to the model $y = ax + b$ using unweighted least-squares regression and the slopes and intercepts of the resulting equations compared to their theoretical values of zero and one, respectively, using Student's two-sided *t*-test at a 0.05 significance level. The calculated values for the slope and intercept for the TDS were 0.982 and 0.078, respectively. The calculated *t*-values for the slope and intercept were 2.325 and 1.097, respectively. Comparison with the tabulated value of 2.306 (8 degrees of freedom, $p = 0.05$)

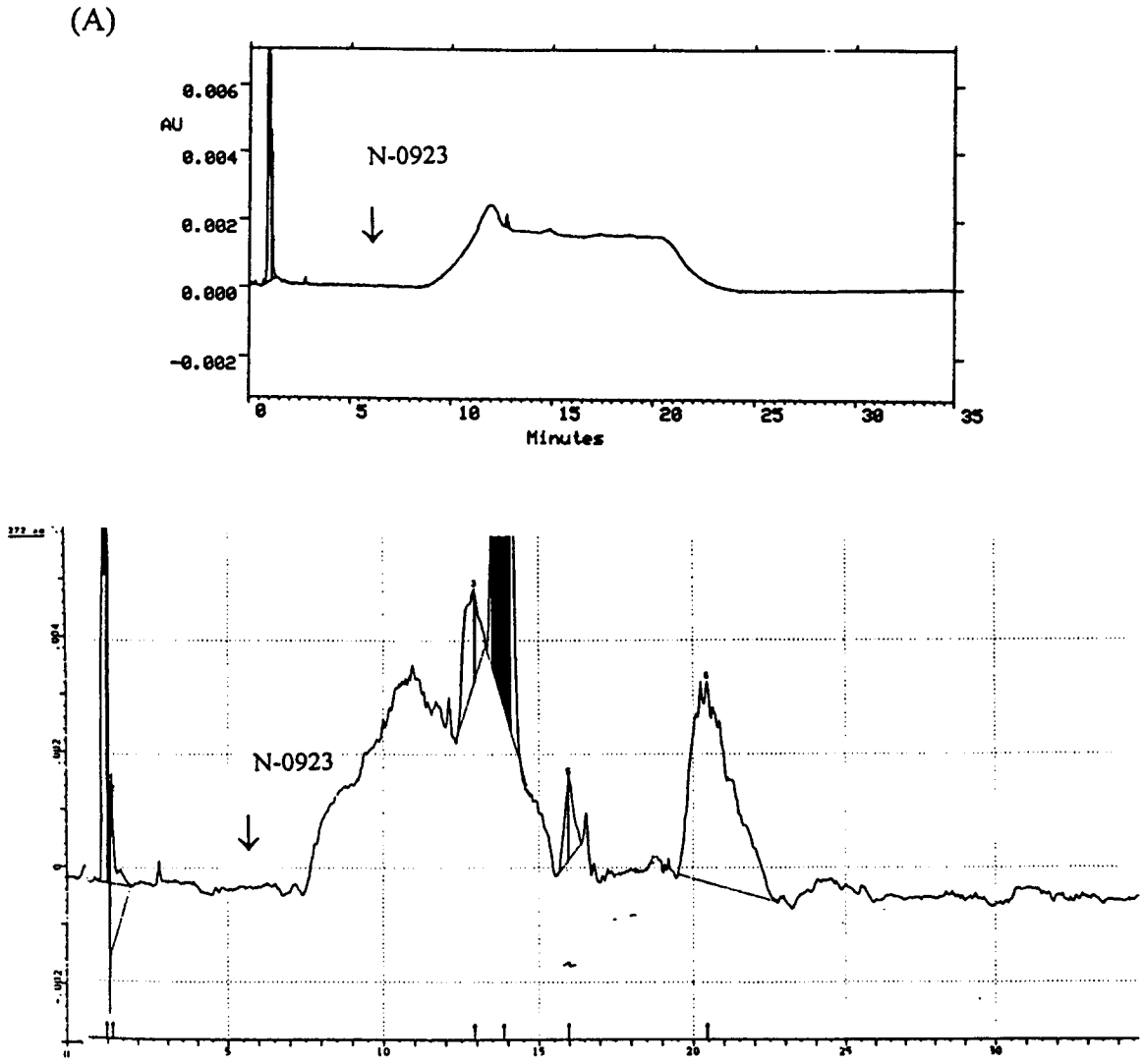

Fig. 3. Chromatograms of N-0923 transdermal placebo system at three times working level (A) and 7-day thermally degraded transdermal placebo system at two times working level (B). The retention time of N-0923 is indicated by arrows.

shows that no significant difference from theory for the intercept can be demonstrated. Although the calculated *t*-value for the slope for N-0923 TDS product was *statistically* significant, due to the high precision of the data and the resulting narrow confidence bands about the fitted lines, the method is considered valid. The mean recovery across the range was $99.2 \pm 1.02\%$. The excellent agreement with theory for assays of active TDS (vide infra) provides further evidence that the procedure gives quantitative recovery. No curvature was evident in the recovery data. Because of the narrow range of interest, high accuracy and precision and good recovery, single point calibration is valid across the evaluated concentration range for the TDS.

3.5. Precision and detection limit

Analysis of 10 individual samples of I TDS produced a mean assay of 9.95 mg/patch (99.5% of label claim) with 5.6% R.S.D. The method shows fully acceptable within-day precision and

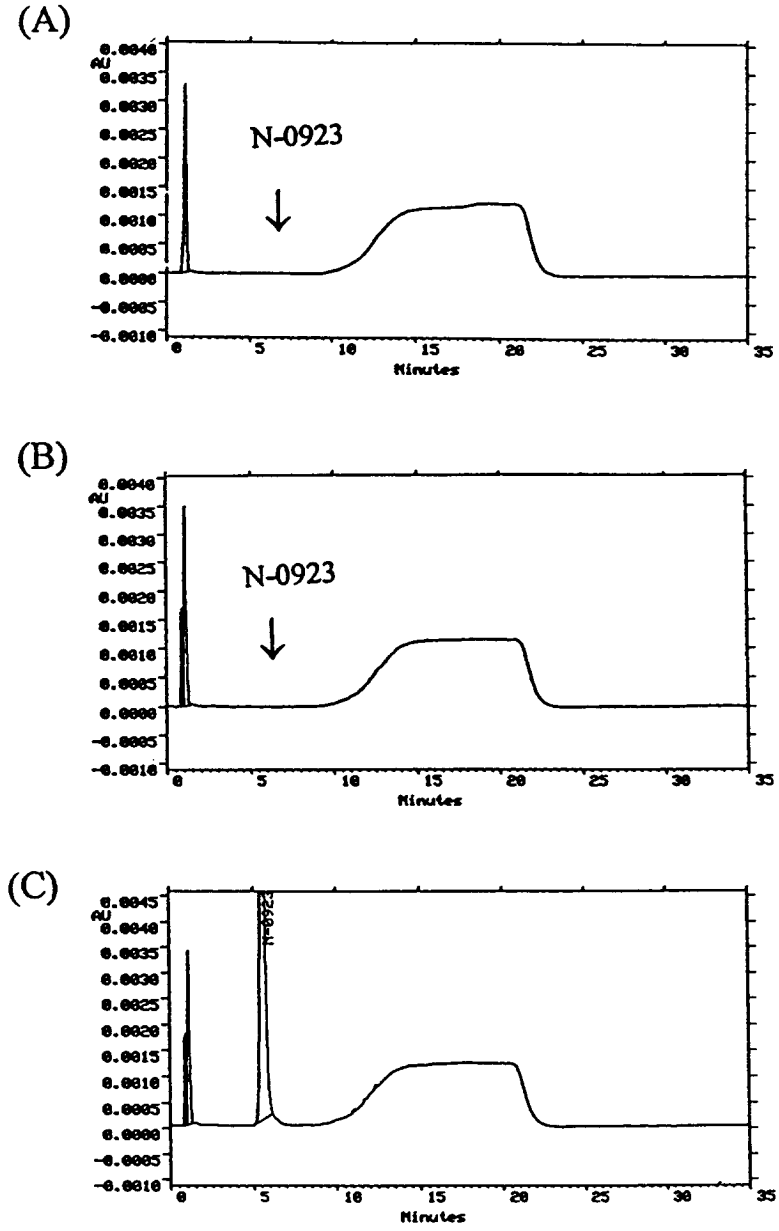

Fig. 4. N-0923 transdermal delivery system impurity screen, citric acid blank (A), placebo (B), active (C). The retention time of N-0923 is indicated by arrows.

accuracy. Analysis of four injections of the working standard produced an R.S.D. of ≤1.0%.

The ability of the method to detect small amounts of drug or drug compounds was evaluated by analysis of a standard solution (0.1 μg/ml) corresponding to 0.05% of the working range (200 μg/ml).

4. Conclusions

A convenient method for analysis of I in a transdermal delivery system using liquid–liquid extraction and gradient reversed-phase HPLC with internal standard quantitation has been developed and validated. Extension of this ap-

Table 2
Linearity of recovery results for N-0923 from a Cygnus transdermal delivery system

Added (mg)	Found (mg)	Recovery (%)		
		Individual values	Mean	R.S.D.
14.77	14.72	99.6	99.1	0.7
	14.56	98.6		
12.31	12.11	98.4	97.9	0.7
	12.04	97.8		
9.85	9.78	99.3	99.75	0.6
	9.87	100.2		
7.38	7.33	99.3	99.0	0.3
	7.30	98.8		
4.92	4.99	101.4	100.3	1.5
	4.88	99.2		

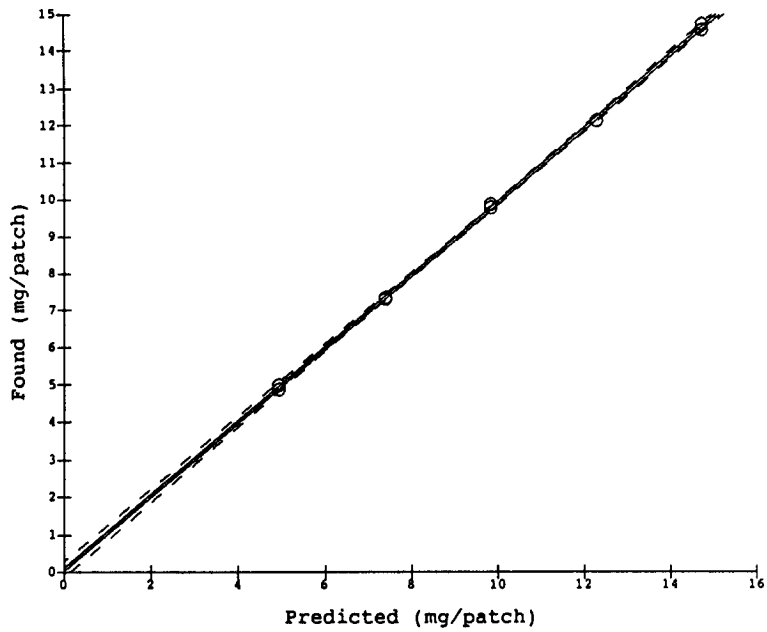

Fig. 5. N-0923 transdermal delivery system spiked placebo recovery data (dashed lines indicate 95% confidence bands).

proach to other basic drugs in similar TDS matrices should be straightforward.

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