Quantitative Determination of Phencyclidine in Pigmented and Nonpigmented Hair by Ion-Trap Mass Spectrometry

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Abstract

A sensitive and specific method has been developed for the quantitative analysis of phencyclidine (PCP) in pigmented and nonpigmented rat hair. After the addition of PCP-d₅ as the internal standard, hair samples (10 mg) were digested overnight in 1N NaOH at 30°C. Digested solutions were then extracted using a solid-phase procedure with Bond Elut Certify™ extraction columns. Reconstituted extracts were analyzed on a Finnigan ion trap (Magnum™) mass spectrometer in the electron ionization mode using helium as the carrier gas, and a DB-5 MS (30 m x 0.25-mm i.d.; 25-μm film thickness) capillary column. The assay is linear from 0.1 to 50 ng/mg with a correlation coefficient of > 0.99 and is capable of detecting 25 pg of PCP on column. The accuracy of this assay was estimated using fortified hair standards at PCP concentrations of 0.5 and 10 ng/mg. Intra-assay coefficients of variation were determined to be less than 6% at 0.5, 2, and 10 ng/mg. Interassay coefficients of variation were determined to be less than 15% at 0.5, 2, and 10 ng/mg. The method has been used to evaluate PCP incorporation into Long-Evans rat hair but could also be used to evaluate the incorporation of PCP into human hair. Male rats were shaved prior to dosing such that both pigmented and nonpigmented hair was collected. Animals were administered 12 mg/kg PCP by intraperitoneal injection daily for five days. Fourteen days after the first dose, pigmented and nonpigmented hair were collected and analyzed for PCP. The mean plus or minus the standard error of the mean (n = 5) concentrations of PCP in pigmented and nonpigmented hair were 14.33 ± 1.43 ng/mg of hair and 0.47 ± 0.04 ng/mg of hair, respectively. This method is also being used to evaluate PCP as a model xenobiotic for studies of the incorporation of xenobiotics into hair.

Introduction

Hair is an increasingly popular matrix for measuring exposure to xenobiotics, particularly drugs of abuse. Recently, it has been suggested that pigmentation may play an important role in the incorporation of certain drugs into hair (1–3). An understanding of the mechanisms involved in the incorporation of drugs into hair and the potential influence of pigmentation is critical in interpreting drug concentrations in hair. Phencyclidine (PCP) is a drug of abuse that has previously been shown to be incorporated into hair (4–8) and may be a useful model xenobiotic for studying mechanisms of incorporation into hair.

Animal models that permit controlled administration of xenobiotics and collection of both pigmented and nonpigmented hair exist and can be used to elucidate mechanisms involved in the disposition of drugs into hair with respect to pigmentation. The Long-Evans (LE) rat produces both pigmented and nonpigmented hair, thus eliminating any potential bias due to strain differences when interpreting data from pigmented and nonpigmented animals.

This paper reports the validation of a sensitive and specific analytical method for the quantitation of PCP in hair using gas chromatography–ion-trap mass spectrometry (GC–ion-trap MS). The method is relatively simple and provides sufficient sensitivity to detect 25 pg of PCP on column. The application of this method to quantitate incorporated PCP in pigmented and nonpigmented hair from LE rats is described.

Materials and Methods

Chemicals and reagents

PCP (1 mg/mL in methanol) and PCP-d₅ (100 μg/mL in methanol) were obtained from Radian Corp. (Austin, TX) for preparation of standard curves; PCP (1 mg/mL in methanol) and PCP HCl powder were obtained from Sigma Chemical (St. Louis, MO) for preparing quality control samples for accuracy evaluation and for preparation of dosing solutions, respectively. Bond Elut Certify™ solid-phase extraction (SPE) columns were obtained from Varian (Harbor City, CA); methanol and ethyl acetate (HPLC grade) were obtained from Burdick and Jackson (Muskegon, MI); ultra-high purity helium (99.99%) was obtained from Mountain Airgas (Salt Lake City, UT).
All other reagent-grade chemicals were obtained from Mallinckrodt Chemical Works (St. Louis, MO). All drug solutions were prepared in HPLC-grade methanol, and HPLC-grade solvents were used in all extractions.

Standards and solutions
Stock solutions containing PCP (100 ng/mL) in methanol for standard curves and quality control samples were prepared and stored at -20°C. From these stock solutions, working solutions were prepared at 0.1, 1, and 10 ng/mL for preparation of daily calibration standards and quality control samples. Daily standard curves were obtained by analyzing hair samples (10 mg) fortified with PCP at 0.1, 0.2, 0.3, 0.5, 0.8, 1, 2, 3, 5, 10, 25, and 50 ng/mg hair. Quality control samples (0.5 and 10 ng/mg hair) were prepared daily from stock sources of reference solutions different from those used to prepare standard curves; drug-free hair was extracted and analyzed as a negative control.

Sample digestion and extraction
After the addition of 5 ng/mg PCP-d5 (internal standard) to 10 mg of hair, samples were completely solubilized (digested) overnight with 1N NaOH at 30°C. The pH of the digests was adjusted to 6.0 with the addition of 6N HCl and 0.1M (w/v) KH2PO4 (pH 6.0). The digest solutions were extracted using Bond-Elut Certify SPE columns according to the manufacturer's specifications with minor modifications. The application, rinse, and elution steps were performed using centrifugation (200-1000 rpm) rather than a vacuum manifold. Columns were conditioned with methanol and 0.1M KH2PO4 (w/v, pH 6.0) (200 rpm for 2 min); the digest solution was applied to the column (400 rpm for 5 min) followed by 1 mL of 1M acetic acid (v/v) (750 rpm for 5 min) and 5 mL methanol (1000 rpm for 5 min). PCP was then eluted with 4 mL of 2% ammonium hydroxide in ethyl acetate (v/v) (200 rpm for 5 min). The eluate solutions were evaporated to dryness at room temperature under air, and the dried extracts were then reconstituted in 40 μL ethyl acetate prior to analysis by GC–MS.

GC–MS analysis
Electron ionization mass spectrometric analysis of reconstituted extracts was performed on a Finnigan Magnum™ (San Jose, CA) ion-trap mass spectrometer coupled to a Varian 3400 gas chromatograph equipped with a Varian 1075 injector and a J&W Scientific (Folsom, CA) DB-5 MS capillary column (30 m x 0.25-mm i.d.; 0.25-μm film thickness). Ultra-high purity helium was used as a carrier gas with a head pressure of 9 psi; 1 μL of reconstituted extract was injected while the instrument was in the splitless mode. The column temperature was held at 125°C for 0.5 min, increased to 275°C at 20°C/min, and then held at 275°C for 0.5 min. The injector was maintained at 200°C; the transfer line was maintained at 250°C; and the manifold was maintained at 200°C. The filament emission current was 12μA; the electron multiplier voltage was 1700V. The electron ionization mode was used with the automatic gain control on and 300 ms ionization time. GC–MS analysis of the mass range 175–275 amu was performed at 0.5 s/scan for all extracts.

Animal protocols
Male Long-Evans rats (approximately 150 g) were obtained from Harlan-Sprague Dawley (Indianapolis, IN) and maintained in an environmentally controlled room with a 12-h
light–dark cycle and free access to food and water. Animals were housed individually in wire hanging cages to prevent contamination from the urine or saliva of other rats and the bedding. PCP hydrochloride dissolved in normal saline (10 mg/mL) was administered by intraperitoneal (i.p.) injection once daily at a dose of 12 mg/kg (n = 5) for 5 days. Before dosing, the animals were shaved to the skin in regions on the back containing only pigmented hair and regions containing only non-pigmented hair with an electric animal shaver. These shaved areas were allowed to grow hair during the five days of drug administration and were resheared 14 days after beginning drug administration. Hair samples were again collected from the same areas 28 days after drug administration (day 28). Hair was stored at -20°C before digestion and extraction. To evaluate whether or not skin was collected during shaving, hair was examined under high power magnification for the presence of keratinocytes. A negligible number of skin cells were observed (approximately 1 cell/100 hairs). Therefore, it seems unlikely that drug in skin cells substantially contributes to the reported hair concentrations.

### Results and Discussion

#### Analytical method

Though methods for the analysis of PCP have been reported in urine and plasma, only a few have discussed the analysis of PCP in hair (4–8); and though ion-trap MS methods for the analysis of PCP have been reported (9,10), hair was not the matrix of interest. The purpose of this study was to develop a sensitive and specific assay for the quantitation of PCP in hair using GC–ion-trap MS. SPE was chosen because it has the advantage of reducing background signal for ion-trap MS and offers increased simplicity over liquid–liquid techniques used in our laboratory.

Figure 1 shows ion chromatograms from an extracted blank rat hair sample. Nonpigmented hair was selected for preparation of standard curves and quality control samples. However, it should be noted that no differences were observed in background noise from either pigmented or nonpigmented hair. Figure 2 shows ion chromatograms from an extracted hair standard fortified with 0.1 ng/mg PCP. The peak shape is acceptable and shows a signal-to-noise ratio of 6.9. A typical standard curve ranging from 0.1 to 25 ng/mg is shown in Figure 3. The base peaks for PCP and PCP-d5 are m/z 200 and m/z 205, respectively, and were used for quantitation of samples. Peak height ratios (PCP/PCP-d5) of the base peaks (m/z 200/205) were calculated for each standard and plotted against the target concentration of the standard. Simple regression correlation coefficients for these standard curves were calculated using CricketetGraph™ (Malvern, PA) software. Correlation coefficients were routinely greater than 0.99. The assay was linear from 0.1 to 50 ng/mg, but for daily quantitation of data, it was only necessary to have standards linear to 25 ng/mg hair.

Alternatively, the Magnum quantitation software could be used. Changes in retention time (Art) were never greater than 3%; the masses selected by the quantitation software for identifying PCP in the quantitation spectrum match were m/z 186, 200, 201, 242, 243, 244; and for PCP-d5 were m/z 176, 177, 188, 190, 191, 204, 205, 206, 207, 246, 247, 248, 249, 250. These masses were established by injecting unextracted standard

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**Table 1. Precision and Accuracy for Analysis of Hair Fortified with PCP**

<table>
<thead>
<tr>
<th>Target Concentration (ng/mg)</th>
<th>Mean Concentration (ng/mg)</th>
<th>% CV*</th>
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</thead>
<tbody>
<tr>
<td>Precision</td>
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<tr>
<td>Intra-assay</td>
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</tr>
<tr>
<td>0.5</td>
<td>0.49</td>
<td>3.70</td>
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<td>1.59</td>
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<tr>
<td>10.0</td>
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<td>5.19</td>
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<tr>
<td>Inter-assay</td>
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<td></td>
</tr>
<tr>
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<td>0.50</td>
<td>4.40</td>
</tr>
<tr>
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<tr>
<td>10.0</td>
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</tr>
<tr>
<td>Accuracy</td>
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<td></td>
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<tr>
<td>0.5</td>
<td>0.47</td>
<td>5.73</td>
</tr>
<tr>
<td>10.0</td>
<td>9.56</td>
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</tr>
</tbody>
</table>

*CV = Coefficient of variation
were negative for PCP (data not shown). Figure 4 shows the total recovery for PCP in fortified hair at 2 ng/mg was determined to be within 6% of the target values with coefficients of variation of less than 6%.

Toxicokinetic differences that may arise due to rat strain. Predose and day 28 hair collections were analyzed and, as expected, were negative for PCP (data not shown). Figure 4 shows the mean plus or minus the standard error of the mean (SEM) (n = 5) concentration of PCP measured in pigmented and non-pigmented hair from LE rats that were administered PCP.

PCP from stock solutions separate from those used for the standard curve. From four or five separate analyses each at 0.5 and 10 ng/mg hair, respectively. Interassay precision was determined by comparing calculated PCP concentrations from drug-free hair fortified with PCP from stock solutions separate from those used for the standard curve. From four or five separate analyses each at 0.5 and 10 ng/mg hair. Accuracy was calculated to be within 6% of the target values with coefficients of variation of less than 6%. Total recovery for PCP in fortified hair at 2 ng/mg was determined to be greater than 80%.

Figure 4. The concentration of PCP in pigmented and nonpigmented hair collected from five LE rats fourteen days after the initiation of a dosing protocol consisting of 12 mg/kg PCP in saline daily for five days by intraperitoneal injection. 10 mg of each hair type was prepared and analyzed using the described analytical method. Error bars represent the standard error of the mean (SEM). *Indicates a significant difference (p < 0.05) as determined by the Mann-Whitney U nonparametric test.

Analysis of PCP in pigmented and nonpigmented rat hair

The method described was used for a preliminary study of PCP as a model compound to determine the role of pigmentation in the incorporation of xenobiotics into hair. The Long-Evans rat is useful for this purpose as it has the advantage of permitting collection of pigmented and nonpigmented hair types from the same animal, eliminating potential pharma-cokinetic differences that may arise due to rat strain. Predose and day 28 hair collections were analyzed and, as expected, were negative for PCP (data not shown). Figure 4 shows the mean plus or minus the standard error of the mean (SEM) (n = 5) concentration of PCP measured in pigmented and non-pigmented hair from LE rats that were administered PCP. In pigmented hair, the PCP concentration was 14.3 ± 1.43 ng/mg, whereas in nonpigmented hair the PCP concentration was 0.5 ± 0.04 ng/mg. The 30-fold difference in PCP concentration between pigmented and nonpigmented hair corresponds with the approximately 46-fold difference observed for codeine in our laboratory.

Recently, Sakamoto et al. (7) reported 3.34 ng/mg PCP in Dark Agouti (DA) rat hair after 10 days of administration of PCP at a dose of 0.5 mg/kg corresponding to a total dose of 5 mg/kg. The total dose we report here (60 mg/kg) is 12 times greater than that used by Sakamoto and co-workers. However, the pigmented hair concentrations in our study were only 4 times greater (14.3 ng/mg vs. 3.3 ng/mg). Possible explanations for this observation may be the different dosing protocols, the saturation of drug incorporation in our experiments, or the different strains of rats used in each experiment. DA rats are known to be deficient in the cytochrome P450-mediated oxidation pathway shown to generate metabolites of PCP (11). We did not measure plasma concentrations in this preliminary study to determine if they were 12 times greater than those reported by Sakamoto, et al. (7). The differences in these two studies demonstrate the importance of considering plasma concentration and strain difference when interpreting data regarding drug incorporation into hair.

Conclusion

This paper describes a sensitive and specific method for the analysis of PCP in hair using GC-ion-trap MS. The assay has a limit of quantitation of 0.1 ng/mg and is sensitive enough to detect 25 pg of PCP on column. The data presented indicate that PCP is incorporated preferentially into the pigmented hair of Long-Evans rats, an animal model useful in evaluating the influence of pigmentation on drug incorporation into hair. This method is currently being used to quantitate PCP in rat hair in an effort to establish some of the mechanisms involved in the incorporation of xenobiotics into hair.

Acknowledgment

This work was supported by NIDA grant #DA07820. The authors would like to thank Gerald G. Kreuger, M.D., Department of Dermatology, University of Utah Health Sciences Center, for his assistance in examining collected hair for keratinocytes.

References


Manuscript received March 26, 1996; revision received May 17, 1996.