Journal of Chromatography B, 878 (2010) 2548–2553

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

# Method for measurement of the quaternary amine compounds paraquat and diquat in human urine using high-performance liquid chromatography–tandem mass spectrometry☆,☆☆

Ralph D. Whitehead Jr.[a], M. Angela Montesano[a], Nayana K. Jayatilaka[a], Brian Buckley[b], Bozena Winnik[b], Larry L. Needham[a], Dana Boyd Barr[a,*]

[a] Centers for Disease Control and Prevention (CDC), National Center for Environmental Health, Division of Laboratory Sciences, Atlanta, GA, USA
[b] Environmental and Occupational Health Sciences Institute, Rutgers University, Piscataway, NJ, USA

ARTICLE INFO

*Article history:*
Received 4 June 2009
Accepted 21 September 2009
Available online 25 September 2009

*Keywords:*
Paraquat
Diquat
Quaternary amines
Urine
HPLC
Tandem mass spectrometry

ABSTRACT

We have developed a highly selective and sensitive analytical method to quantify paraquat and diquat by use of high-performance liquid chromatography-tandem mass spectrometry (HPLC–MS/MS). The sample preparation includes solid phase extraction that uses weak cation exchange cartridges. These highly charged dual quaternary amines were not retained by standard reversed phase columns, but they could be adequately separated through HPLC with a HILIC column. The detection was carried out with a triple quadrupole mass spectrometer with an electrospray ionization probe in positive ion mode in multiple reaction monitoring. Repeated analysis in human urine samples spiked with low (5 ng/ml), medium (15 ng/ml), and high (30 ng/ml) concentrations of the analytes yielded relative standard deviations of less than 9%. The extraction efficiencies ranged from 77.7% to 94.2%. The limits of detection were in the range of 1 ng/ml.



## 1. Introduction

Paraquat (PQ) and diquat (DQ) are quaternary ammonium compounds widely used as non-selective contact herbicides. PQ and DQ are used as desiccants and defoliants for the control of weeds and grasses in fruit orchards, plantations that grow potatoes, cotton, and hops, and aquatic environments. The commercial herbicide formulations usually contain diquat dibromide and paraquat dichloride [1,2]. PQ and DQ are suitable for many agricultural uses because of their high solubility in water, their low production of vapors during application, and their ability to bind to soil [3]. Fig. 1 shows the structures of these two target analytes.

Many incidents of accidental and intentional exposures to these compounds have been reported [1,2,4]. The primary route of exposure to PQ and DQ is ingestion, although in rare occurrences, inhalation and dermal exposure has caused toxic effects. Upon PQ absorption, the compound primarily accumulates in the lungs, resulting in acute pulmonary distress, but it also has drastic effects on the gastrointestinal tract, the kidneys, the liver, and the heart. DQ also accumulates in the lungs, liver, and kidneys, but to a lesser extent than PQ [5–10]. Both PQ and DQ target the central nervous system, with most effects seen in the brain stem [8,11,12]. PQ is believed to be associated with Parkinson's Disease because of its deleterious effects on the dopaminergic neurons [13–16].

Gas chromatography (GC)– and high-performance liquid chromatography(HPLC)–mass spectrometry analysis (MS) have been used for the measurement of PQ and DQ in different matrices, including water [17–21], agricultural products [22,23] and biological samples [24–29]. In the GC–MS methods, a chemical reduction of PQ and DQ was performed by use of such compounds as sodium tetrahydridoborate in order to obtain more volatile compounds for the gas chromatographic analysis [24,25,28,29]. Because PQ and DQ are doubly charged cationic species in solution, HPLC–MS using electrospray ionization has become the technique of choice for the analysis of these analytes [17–23].

Previous HPLC–MS electrospray methods have used mobile phases containing ion-pairing reagents such as heptafluorobutyric (HFBA), pentafluoropropionic (PFPA), or trifluoroacetic (TFA) acids in order to chromatograph the extremely polar PQ and DQ on reverse-phase silica-based C8 or C18 columns [17–23,28]. The addition of salts to the mobile phase containing the ion-pairing reagents

☆ This paper is part of the special issue "Biological Monitoring and Analytical Toxicology in Occupational and Environmental Medicine", Michael Bader and Thomas Göen (Guest Editors).
☆☆ The views expressed in this paper are those of the authors and do not necessarily reflect the views or policies of the Centers for Disease Control and Prevention.
* Corresponding author at: CDC, 4770 Buford Hwy, Mailstop F17, Atlanta, GA 30341, USA. Tel.: +1 770 488 7886; fax: +1 770 488 4967.
*E-mail address:* dbarr@cdc.gov (D.B. Barr).

1570-0232/$ – see front matter © 2009 Published by Elsevier B.V.
doi:10.1016/j.jchromb.2009.09.029

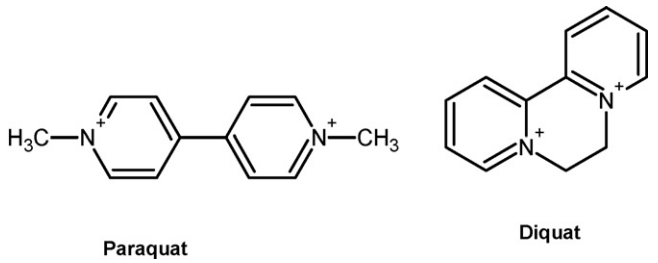


**Fig. 1.** Structures of the target analytes.

has been shown to improve the separation and resolution of PQ and DQ [26,27]. However, the presence of ion-pairing reagents in the mobile phase decreases the sensitivity of the methods because these reagents have the effect of suppressing the formation of ions, thus decreasing the quantity of ions that reaches the mass spectrometer detector [30,31]. Only one HPLC–MS method has been published using urine as a matrix with limits of detection (LODs) in the 5–10 ng/ml range.

In the present study, we describe an analytical method for measuring PQ and DQ in urine with improved sensitivity and selectivity that does not use ion-pairing reagents [17–23,28]. We used a HILIC (hydrophilic interaction chromatography) column for the HPLC separation of these compounds, followed by electrospray ionization-tandem mass spectrometry (ESI-MS/MS). The sample preparation was simple, and the method employed a solid phase extraction using weak cation exchange cartridges. Our method is simpler than those previously published for analyzing biological matrices, allowing for better precision, and the separation we performed without the use of ion-pairing agents gives our method better sensitivity than previously published methods.

## 2. Materials and methods

### 2.1. Chemicals

The native standards of paraquat dichloride x-hydrate (1,1′-dimethyl-4,4′-dipyridinium dichloride hydrate) and diquat dibromide monohydrate (1,1′-ethylene-2,2′-bipyridinium dibromide) were purchased from Sigma–Aldrich (St. Louis, MO) and Chem Service, Inc. (West Chester, PA, USA), respectively. The labeled standards paraquat-ring-$d_8$·2HCl (PQ-label) and diquat dibromide-1,1′-ethylene-$d_4$ (DQ-label) were custom-synthesized by Cambridge Isotope Laboratories (Andover, MA, USA).

All solvents used were of analytical grade. Acetonitrile and methanol were purchased from Tedia Company, Inc. (Fairfield, OH). Formic acid was purchased from Acros Organics (Morris Plains, NJ). Deuterium oxide ($D_2O$) was purchased from Sigma–Aldrich (St. Louis, MO). Ammonium formate was purchased from Sigma–Aldrich (St. Louis, MO). Deionized water was organically and biologically purified by use of an Aqua Solutions, Inc. water system (Jasper, GA).

### 2.2. Standard and internal standard preparation

The stock solutions of the native paraquat (PQ) and diquat (DQ) were prepared by weighing out approximately 3–4 mg of each analyte and dissolving in 10 ml of deionized water. Stock solutions were stored at −70 °C. Ten working standard solutions, each a mixture of an equal concentration of all the analytes, covering a range of 10–8000 ng/ml, were prepared by performing serial dilutions of the 8000 ng/ml solution by deionized water. The working standard solutions were stored at −20 °C. Nine calibration standards were made by adding the working stock solutions to blank urine covering a range from 0.125 to 50 ng/ml (50, 25, 10, 5, 2.5, 1, 0.5, 0.25, and 0.125 ng/ml). The calibration standards were made freshly before each analytical run. The stability of stock and working standard solutions in water was determined by monitoring peak intensity for each standard over time in the analytical runs.

The combined labeled internal standard stock solution of PQ-label and DQ-label was prepared by weighing approximately 1.5 mg of each isotopically labeled analyte into a 10 ml volumetric flask and dissolving with $D_2O$, which was used to prevent a D-H-exchange. The stock solution was stored at −70 °C. An internal standard working solution mixture containing the labeled analytes was prepared at 1480 ng/ml in $D_2O$ and stored at −20 °C.

### 2.3. Quality control materials

Urine was collected from multiple (>15) donors, combined, diluted with water (1:1, v/v) to reduce endogenous levels of the analytes of interest, and mixed overnight at 4 °C. Our protocol for anonymous collection of urine was reviewed and approved by CDC's Institutional Review Board (IRB). The urine pool was pressure-filtered with a 0.2-μm filter capsule and divided into four pools. The first pool (QCL), the second pool (QCM), and the third pool (QCH) were spiked with the native standard stock solution to yield concentrations of 5, 15 and 30 ng/ml, respectively. The fourth pool was not spiked. After being screened for possible endogenous analytes, the fourth pool was used as matrix material for calibration standards and blanks.

### 2.4. Sample preparation

Each urine sample (2 ml) was pipetted into a 15 ml vial and spiked with 25 μl of the labeled internal standard working solution to give a urinary concentration of 18.5 ng/ml. The urine samples were vortex-mixed. Strata-X-CW 33 μm polymeric 3 ml weak cation cartridges (Phenomenex, Torrance, CA) were conditioned with 1 ml methanol, followed by 1 ml deionized water. The samples were loaded onto the cartridges. The cartridges were then washed with 1 mL of 5% methanol in deionized water (v/v). The cartridges were eluted with 10% formic acid in acetonitrile (v/v), and the elutant was collected in 15 ml centrifuge tubes. The samples were concentrated through use of a Turbovap LV (Zymark, Hopkinton, MA) at 40 °C and 10 psi of nitrogen. Acetonitrile (300 μl) was added to each tube, followed by vortex mixing to rinse the tube, and then concentrated to dryness. The residues were reconstituted with 50 μl of mobile phase and transferred to auto-injection vials.

### 2.5. Chromatography and mass spectrometry conditions

Chromatographic separation was performed by use of a Surveyor HPLC system (ThermoFisher Scientific, San Jose, CA, USA) composed of an autosampler and an HPLC pump. The column used was an Atlantis® HILIC Silica, 2.1 mm × 150 mm, 5.0 μm (Waters Corporation, Milford, MA). The analytes were separated with isocratic elution by using 60% 250 mM ammonium formate in deionized water, pH 3.7, and 40% acetonitrile for 10 min. The flow rate was 400 μl/min and the injection volume was 5 μl. The divert valve was programmed to go to waste for the first 2 min and the last 30 s of each run. The Surveyor HPLC pump pressure was the maximum 400 bar.

For the MS/MS analysis, a TSQ Quantum Ultra triple quadrupole mass spectrometer (ThermoFisher Scientific, San Jose, CA, USA) was used. The instrument was operated with an ESI (electrospray ionization) source. The source was operated in positive ion mode using multiple reaction monitoring (MRM). The instrument parameters were as follows: sheath gas pressure 33, auxiliary gas pressure 5,

capillary temperature 350 °C, spray voltage 3800 V, and collision gas pressure 1.5 mTorr.

### 2.6. Extraction efficiency

The extraction recovery of the analytes was determined at two concentrations, 25 and 100 ng/ml, by spiking five blank urine samples with the appropriate standard concentration and processing according to the method. Five additional blank urine samples (unspiked) were processed concurrently. Before the evaporation steps, all of the samples were spiked with a known amount of labeled internal standard to correct for instrument variation. The samples that were not spiked before preparation were then spiked with the appropriate native standard to serve as control samples representative of 100% recovery. After evaporating and reconstituting, the samples were analyzed. This procedure was repeated two more times, and the recovery for all three runs was calculated by comparing the responses of the blank urine samples spiked before extraction to the responses of the blank urine samples spiked after extraction.

### 2.7. Quantification and quality control of analytical runs

Just before each analytical run, calibration standards were prepared by diluting the working standard stock solutions in blank urine. The concentrations of the nine calibration standards ranged from 0.125 to 50 ng/ml for each of the analytes. To each run was added the nine calibration samples, two sets of three quality control samples (QCL, QCM, and QCH), and one blank urine sample; these were extracted and analyzed in parallel with the unknown samples. The area of the analyte divided by the area of the internal standard was plotted against the concentration of the sample to derive a calibration plot. The best fit line of a linear regression analysis of the plot was used to derive an equation from which unknown sample concentrations could be calculated.

All QC pools were characterized before use to determine the mean and 99th and 95th control limits by consecutively analyzing at least 20 samples from each QC pool. QC samples were analyzed in runs with 2 replicates in 10 runs over 10 days. After establishment of the control limits of the pools, individual QC samples contained within each analytical run were evaluated for validity by use of Westgard multirules [32].

### 2.8. Limits of detection

The LOD was calculated for each analyte as three times the standard deviation of the noise at zero concentration ($3S_0$), where $S_0$ was estimated as the $y$-intercept of a linear regression analysis of a plot of the standard deviation of the four lowest standards versus the expected concentration from seven runs [33]. The calculated LODs were verified by analyzing a sample spiked at the LOD concentration to ensure visual detection of the analytes.

### 2.9. Accuracy

The accuracies, sometimes called relative recoveries, were calculated by spiking blank urine samples at different concentrations and calculating the concentration by this method. A linear regression analysis was performed on a plot of the measured concentrations versus the expected concentrations. A slope of 1.00 was considered 100% accuracy.

### 2.10. Precision

The method precision was determined by calculating the relative standard deviations (RSDs) of repeat measurements of the QC materials at three different concentrations (5, 15, and 30 ng/ml). At least 20 repeat measurements of QC materials were used to determine the method RSDs for each analyte.

### 2.11. Stability and adsorption analyses

The stability of the analytes in matrix was determined by monitoring degradation of the samples stored at different temperatures and times. Blank urine (2 ml) samples were spiked with 25 μl of working solutions to yield a concentration of 10 ng/ml. Samples were stored for 5, 18, or 24 h at temperatures of −20 °C, 4 °C, room temperature (RT), or 37 °C.

To measure the adsorption of analytes onto the storage containers, two different types of container materials (i.e., plastic and glass) were tested. Samples were stored in 15 ml BD® Falcon® polypropylene conical tubes (17 mm × 120 mm) or in 15 ml Kimble® disposable glass conical centrifuge tubes (type 1, snap cap finish), both from Fisher Scientific, Pittsburgh, PA. Samples were prepared in triplicate; just before the samples were processed, a blank urine sample spiked with equal amounts of the analytes was prepared in glass and plastic and processed with the other sample as a zero time storage control. The stability was calculated as a ratio area count at different conditions of storage in relation to zero time storage. If any sample degraded over 10% during the storage time, it was considered unstable at that temperature. Similarly, adsorption onto storage containers was calculated as a percentage loss per type of storage container.

### 2.12. Matrix effects

Urine matrix effects were evaluated by spiking individual urine samples collected from eight different donors and compared with a spiked urine pool sample formed by combining urine from the same eight donors. Urine samples (2 ml) were spiked with 25 μl of working solution to yield a concentration of 100 ng/ml. The urine samples were prepared for analysis according to the procedure already described. Five replicates were analyzed from each urine sample. The replicates were divided in five analytical runs so that only one replicate from each aliquot was analyzed in each analytical run. An aliquot of each urine matrix was screened for possible endogenous analytes.

### 2.13. Cross-comparison of analytical results with independent laboratory and analytical platform

To ensure both the accuracy and the robustness of our analytical platform for determining PQ and DQ concentrations, we conducted a cross-comparison study with an independent laboratory that measures and quantifies PQ and DQ in multiple matrices, including tissue extracts using ion trap MS (ITMS) for a variety of environmental health studies. The analytical core laboratory at the Environmental and Occupational Health Science Institute (EOHSI) at Rutgers University provided us with tissue extract samples that staff members had previously analyzed in their laboratory using established methods [34]. The percentage of agreement between the two platforms was calculated as the slope of a linear regression analysis of a plot of the values obtained from each method.

## 3. Results and discussion

The precursor and product quantification and confirmation ion pairs, the collision offset energy for PQ and DQ on the TSQ Quantum Ultra, and the retention times are summarized in Table 1. PQ and DQ gave the deprotonated precursor molecular ions $[M-H]^+$ at $m/z$ 185 and 183, respectively. The electrospray ions obtained for PQ and DQ are a function of the mobile phase composition. The $m/z$

**Table 1**
The precursor and product ions, the collision energy, and the relative retention time for the native analytes and their labeled internal standard.

| Analyte | Precursor → product | Collision energy (V) | Relative retention time (min) |
|---|---|---|---|
| PQ-Q | 185 → 169 | 28 | 4.99 |
| PQ-C | 185 → 142 | 38 | 5.00 |
| PQ-ISTD | 193 → 178 | 23 | 5.02 |
| DQ-Q | 183 → 157 | 22 | 4.31 |
| DQ-C | 183 → 130 | 32 | 4.32 |
| DQ-ISTD | 185 → 159 | 24 | 5.00 |

Q, quantification ion; C, confirmation ion; ISTD, internal standard.

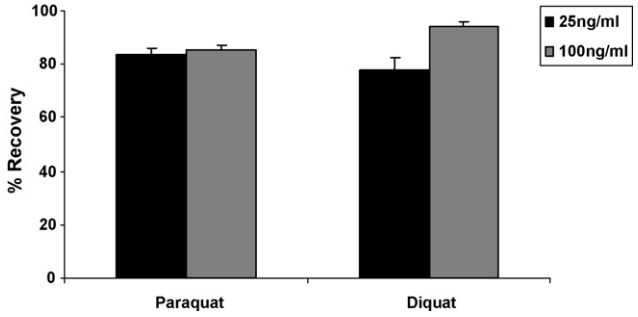


**Fig. 3.** Percentage of recovery of the analytes in two concentrations of spiked urine after the standard clean-up procedure.

185 and 183 are the base peaks with our mobile phase containing ammonium formate. In other methods with different mobile phase composition, the base peaks could be the doubly charged ion [17]. In order to improve selectivity of the analysis, we used the most abundant product ion as a quantification ion and the next most abundant as a confirmation ion.

For the optimization of the electrospray tandem mass spectrometry analysis, two different ion sources, consisting of an ESI and a heated ESI (HESI) source, were used. Data (not shown) comparing the performance of the two probes showed that the signal intensities had the same magnitude for DQ in both probes; however, PQ had higher signal magnitude with the ESI probe under the same resolution conditions. In addition, both analytes showed higher signal-to-noise (S/N) ratios with the ESI probe.

The liquid chromatography was optimized on a HILIC Silica column under isocratic conditions. Good separation and retention of the analytes was achieved in a total run time of 10 min. Analytical separation on HILIC Silica columns of polar analytes is by partitioning and weak cation exchange. This novel column allows the standard separation of analytes with similar physical and chemical properties that cannot typically be separated by standard reversed phase columns. Use of this column enabled us to avoid the use of ion-pairing agents which greatly improved both our chromatography and sensitivity. A typical ion chromatogram of a urine extract spiked with 100 ng/ml of the native analytes and 18.5 ng/ml of the labeled analytes is shown in Fig. 2.

The divert valve of the HPLC system, which directs the solvent flow away from the mass spectrometer's ion source, was programmed to stay open for 2 min after injection to help reduce the chemical background and keep the ion source clean.

The extraction recoveries of the analytes are shown in Fig. 3. The recoveries for PQ were 83.4% (25 ng/ml) and 85.5% (100 ng/ml), and for DQ they were 77.7% (25 ng/ml) and 94.2% (100 ng/ml). In fact, the weak cation exchange cartridges showed good selectivity for these quaternary amines. Both analytes showed a small difference in extraction efficiency between the higher and the lower concen-

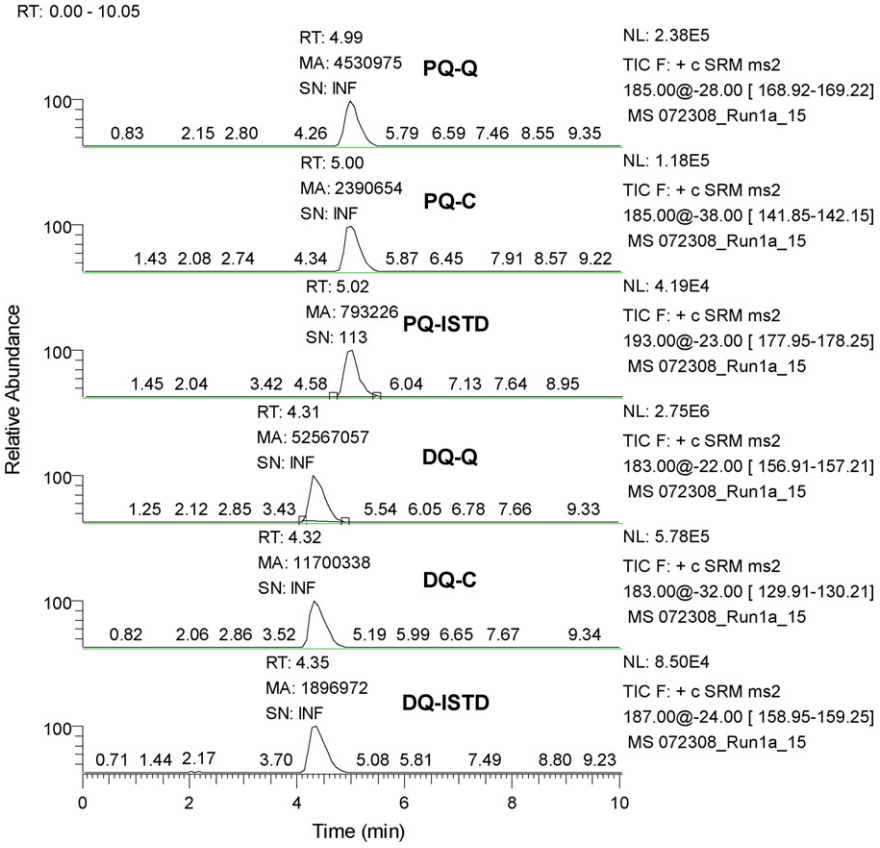


**Fig. 2.** A typical ion chromatogram of native and isotopically labeled analytes (18.5 ng/ml) in spiked urine.

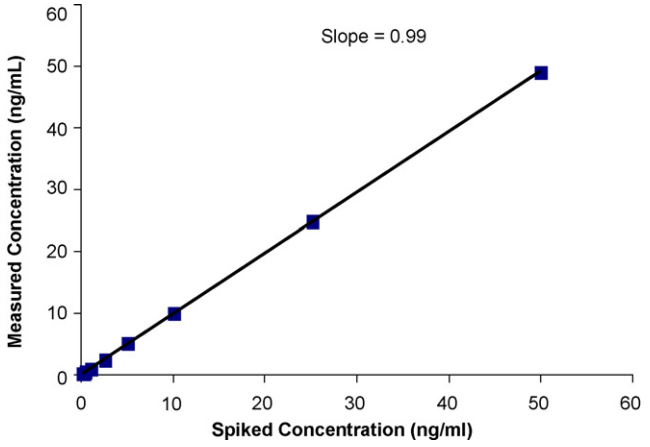


**Fig. 4.** A plot of the spiked concentration versus the measured concentration for paraquat is shown. The slope = 0.99 indicating almost 100% accuracy in the measurements.

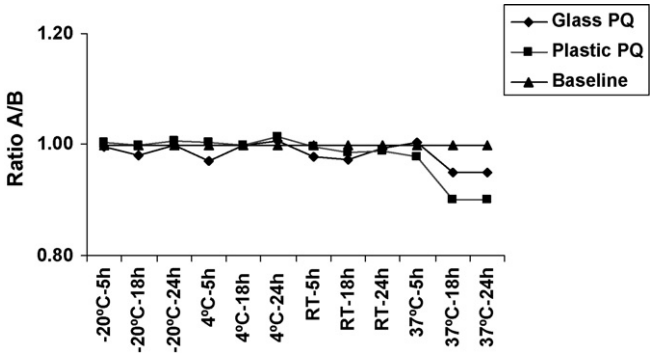


**Fig. 5.** Stability of the analytes. The variation of stability in matrix was calculated as a ratio of area count at different conditions and times of storage (A) versus the area count at storage, $t = 0$ (B).

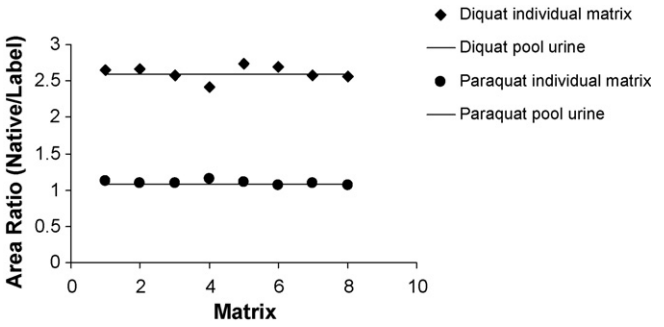


**Fig. 6.** Matrix effects. Urine samples from eight different individual donors and a combined urine sample pool were spiked with the analytes and quantified in five analytical runs. The percentage of variation was calculated as: $(1 - (\text{obtained/expected})) \times 100$, expected = area ratio for the combined urine sample pool; obtained = area ratio for the individual urine sample.

tration. In the isotope dilution technique we used, the individual recovery of each analyte in a sample is automatically corrected so that variable extraction recoveries do not negatively affect the accuracy of the data obtained. A plot of the spiked concentration versus the measured concentration for paraquat, indicative of the accuracy of the method, is shown in Fig. 4.

Calibration plots were linear from 0.125 to 50 ng/ml. The $R^2$ values for the linear regression analyses were 0.999. The error of the data fit about the slope was less than 3%. In addition, for both analytes, slope averages of a linear regression analysis of nine calibration standards of ten runs of calibration curves were calculated, and they are shown in Table 2.

The LODs of the method were 0.63 ng/ml for PQ and 0.13 ng/ml for DQ (Table 2). The LOD calculations were based on seven runs of calibration plots in blank urine matrix. The LODs are lower in magnitude than the LODs previously published for these analytes in urine using LC–MS/MS [26]. Also, the majority of methods reported in the literature for quantification of PQ and DQ in different matrices used only one stage of MS, a less selective analytical technique than the MS/MS that we used in this study [17–23]. The method accuracies (relative recoveries) were 99.6% for PQ and 99.5% for DQ. The calculations were based on a slope average of linear regression analyses of plots of calculated concentrations of spiked samples versus the expected concentration of the same samples from ten runs. The precision of our method is shown in Table 2. It is expressed as the RSD of repeated analyses of the QC materials. The QC values were calculated as an average of nine runs, with three at each level in each run. The quality control system is robust, providing consistent values over time and showing that the QC materials and method are stable.

The stability of the analytes under determined storage conditions, encompassing the duration of time from sample collection to sample processing, is very important for the interpretation of toxicological findings. We investigated PQ and DQ stability in urine matrix that was stored in glass or plastic tubes for 5, 18, or 24 h at −20 °C, 4 °C, room temperature, or 37 °C. Overall, the data suggested that the analytes were stable because no degradation was detectable after 24 h under different storage conditions (Fig. 5).

In addition, possible matrix composition effects were also investigated, because individual sample variation in pH and concentrations of salts and biomolecules in urine might affect the sensitivity, selectivity, or even the accuracy of the method [35]. Urine samples collected from eight different donors were individually analyzed and compared with a urine pool combining urine from the same eight donors. The variation of matrix effects was calculated, and the data are shown in Fig. 6. Sample matrix composition had negligible effect on the quantitative analysis. The average among matrix variations for PQ was 2.47% and that for DQ was 2.97%.

Cross-laboratory comparison studies are important to establish accuracy and confidence in the analytical methodology where ref-

**Table 2**
Summary of method specifications on TSQ Quantum Ultra.

| Analyte | LOD, ng/ml (ppb) | Standard curve $R^2$ | Accuracy (%) | QC values | | | RSD | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | QCL | QCM | QCH | QCL | QCM | QCH |
| PQ | 0.63 | 0.9999 | 99.63 | 5.98 | 15.68 | 29.54 | 6.46 | 6.70 | 6.57 |
| DQ | 0.13 | 0.9999 | 99.47 | 5.91 | 16.07 | 30.48 | 8.67 | 4.77 | 8.59 |

LOD: calculated as $3S_0$. Standard deviation at zero concentration ($S_0$) was estimated as the $y$-intercept of a plot of the standard deviation of the five lowest calibration standards from seven runs versus the expected concentration. Standard curve: slope average of a linear regression analysis of nine calibration standards from ten runs. Accuracy: expressed as the percentage of the expected concentration that was quantified from nine runs. QC (quality control) values: average of QCL (low), QCM (medium) and QCH (high) from nine runs. Blank urine pools were spiked with the native standard stock solution to yield a concentration of 5.0 ng/ml (QCL), 15.0 ng/ml (QCM), and 30.0 ng/ml (QCH). RSD: relative standard deviation of the QC values from ten runs.

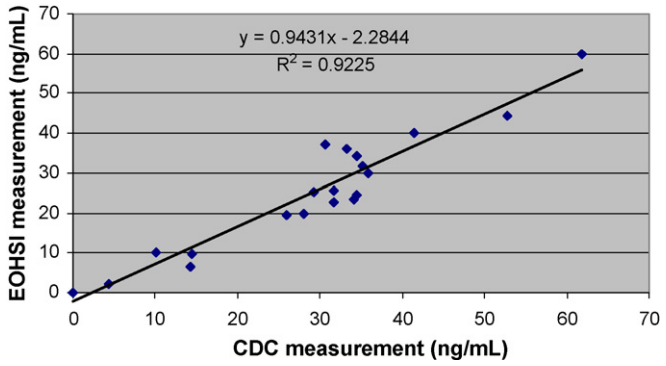


**Fig. 7.** Comparison of measurements of the Environmental and Occupational Science Institute and the Centers for Disease Control and Prevention in biological media extracts. The slope of 0.94 indicates excellent agreement between the two independent laboratories using two different mass spectrometer platforms.

erence standards or proficiency testing programs are lacking or are established in-house [36]. Because such comparison studies are not readily available for PQ or DQ analysis, we sought to find an external laboratory to validate our quantification methods. However, few laboratories are routinely measuring PQ or DQ as a part of their analytical or environmental programs. EOHSI has developed methods to measure PQ in a variety of matrices in support of its environmental and toxicology programs [34]. In tissue extracts that were analyzed by both laboratories, we found 94% agreement among quantified values, providing some assurance for our quantitative measurements (Fig. 7). In addition, these data suggest that our method may be adaptable to matrices other than urine.

## 4. Conclusions

We have developed a precise, sensitive, and reproducible analytical method to quantify PQ and DQ in human urine. The high sensitivity of this method makes it suitable for the measurement of internal doses resulting from incidental and low-level exposures, such as those commonly occurring in environmental exposures.

## Conflict of interest

The authors declare no competing financial interests.

## References

[1] T. Haley, Clin. Toxicol. 14 (1979) 1.
[2] N.M. Sabapathy, Toxicology 91 (1994) 93.
[3] Y. Picó, G. Font, J.C. Moltó, J. Mañes, J. Chromatogr. A 885 (2000) 251.
[4] R.D. Fairshter, A.F. Wilson, Am. J. Med. 59 (1975) 751.
[5] R. Vanholder, F. Colardyn, J. De Reuck, M. Praet, N. Lameire, S. Ringoir, Am. J. Med. 70 (1981) 1267.
[6] P. Mahieu, A. Bonduelle, A. Bernard, A. De Cabooter, M. Gala, A. Hassoun, J. Koenig, R. Lauwerys, Clin. Toxicol. 22 (1984) 363.
[7] J.S. Bus, J.E. Gibson, Environ. Health Perspect. 55 (1984) 37.
[8] G.M. Jones, J.A. Vale, Clin. Toxicol. 38 (2000) 123.
[9] Z.E. Suntres, Toxicology 180 (2002) 65.
[10] T.R. Van Vleet, R.G. Schnellmann, Semin. Nephrol. 23 (2003) 500.
[11] P.J. Landrigan, B. Sonawane, R.N. Butler, L. Trasande, R. Callan, D. Droller, Environ. Health Perspect. 113 (2005) 1230.
[12] S. McCarthy, M. Somayajulu, M. Sikorska, H. Borowy-Borowski, S. Pandey, Toxicol. Appl. Pharmacol. 201 (2004) 21.
[13] D.A. Di Monte, M. Lavasani, A.B. Manning-Bog, Neurotoxicology 23 (2002) 487.
[14] M. Thiruchelvam, O. Prokopenko, D.A. Cory-Slechta, E.R. Richfield, B. Burckley, O. Mirochnitchenko, J. Biol. Chem. 280 (2005) 22530.
[15] R.J. Dinis-Oliveira, F. Remiao, H. Carmo, J.A. Duarte, A. Sanchez Navarro, M.L. Bastos, F. Carvalho, Neurotoxicology 27 (2006) 1110.
[16] M.G. Purisai, A.L. McCormack, S. Cumine, J. Li, M.Z. Isla, D.A. Di, Monte, Neurobiol. Dis. 25 (2007) 392.
[17] V.Y. Taguchi, W.D. Jenkins, P.W. Crozier, D.T. Wang, J. Am. Soc. Mass Spectrom. 9 (1998) 830.
[18] R. Castro, E. Moyano, M.T. Galceran, J. Chromatogr. A 830 (1999) 145.
[19] R. Castro, E. Moyano, M.T. Galceran, J. Chromatogr. A 869 (2000) 441.
[20] R. Castro, E. Moyano, M.T. Galceran, J. Chromatogr. A 914 (2001) 111.
[21] J.L. Vidal, A.B. Vega, F.J. Lopez, A.G. Frenich, J. Chromatogr. A 1050 (2004) 179.
[22] L. Grey, B. Nguyen, P. Yang, J. Chromatogr. A 958 (2002) 25.
[23] M.A. Aramendia, V. Borau, F. Lafont, A. Marinas, J.M. Marinas, J.M. Moreno, J.M. Porras, F.J. Urbano, Food Chem. 97 (2006) 181.
[24] G.H. Draffan, R.A. Clare, D.L. Davies, G. Hawksworth, S. Murray, D.S. Davies, J. Chromatogr. 139 (1977) 311.
[25] S. Kawase, S. Kanno, J. Chromatogr. 283 (1984) 231.
[26] X.P. Lee, T. Kumazawa, M. Fujishiro, C. Hasegawa, T. Arinobu, H. Seno, A. Ishii, K. Sato, J. Mass Spectrom. 39 (2004) 1147.
[27] M.M. Ariffin, R.A. Anderson, J. Chromatogr. B 842 (2006) 91.
[28] R.M. De Almeida, M. Yonamine, J. Chromatogr. B 853 (2007) 260.
[29] N.C. Posecion, E.M. Ostrea, D.M. Bielawski, J. Chromatogr. B 862 (2008) 93.
[30] T.M. Annesley, Clin. Chem. 49 (2003) 1041.
[31] C.R. Mallet, Z. Lu, J.R. Mazzeo, Rapid Commun. Mass Spectrom. 18 (2004) 49.
[32] J.O. Westgard, Basic QC Practices: Training in Statistical Quality Control for Health Care Laboratories, Westgard QC Inc., Madison, WI, 2002.
[33] J.K. Taylor, Quality Assurance of Chemical Measurements, Lewis Publishers, Boca Raton, 1987.
[34] B. Winnik, D.B. Barr, M. Thiruchelvam, E. Richardson, M.A. Montesano, B. Buckley, Anal. Bioanal. Chem. 395 (2009) 195.
[35] S.E. Baker, A.O. Olsson, L.L. Needham, D.B. Barr, Anal. Bioanal. Chem. 383 (2005) 963.
[36] D.B. Barr, G. Leng, E. Berger-Preiss, H.W. Hoppe, G. Weerasekera, W. Gries, S. Gerling, J. Perez, K. Smith, L.L. Needham, J. Angerer, Anal. Bioanal. Chem. 389 (2007) 811.