e-ISSN: 2320-4230, p-ISSN: 2961-6085

Journal of Drug Discovery and Therapeutics

Available Online at www.jddt.in

CODEN: - JDDTBP (Source: - American Chemical Society)

Volume 13, Issue 4; 2025, 18-27

Determination of chlorpyrifos and its metabolites in cells and culture media by liquid chromatography-electro spray ionization tandem mass spectrometry (lc-ms/ms)

Basheer Ahmmad Shaik[1], Arun Kumar Sharma[1]

[1]SunRise University, Alwar Rajasthan

Received: 11-03-2025 / Revised: 10-04-2025 / Accepted: 23-05-2025
Corresponding author: Basheer Ahmmad Shaik
Conflict of interest: No conflict of interest.

Abstract:
A sensitive and specific method was developed for the simultaneous quantification of chlorpyrifos (CPF), its active metabolite chlorpyrifos oxon (CPO), and the detoxified product 3,5,6-trichloro-2-pyridinol (TCP). Sample preparation involved liquid-liquid extraction for culture media samples and protein precipitation for cell samples. Detection was performed using LC-MS/MS with multiple reaction monitoring (MRM) in positive ion mode for CPF and CPO, and selected ion recording (SIR) in negative ion mode for TCP. The method demonstrated linear ranges of 5–500 ng/mL for CPF, 0.2–20 ng/mL for CPO, and 20–2000 ng/mL for TCP in media samples, and 0.5–50 ng/million cells for CPF, 0.02–2 ng/million cells for CPO, and 2–200 ng/million cells for TCP in cell samples. The method was fully validated for selectivity, linearity, precision, accuracy, recovery, stability, and dilution integrity. It has been successfully applied to investigate the neurotoxicity and metabolism of chlorpyrifos in a human neuronal cell model.

Keywords: Chlorpyrifos, Chlorpyrifos Oxon, TCP, Neuron, Metabolism, Neurotoxicity, Culture Media, LC-MS/MS

Introduction

Chlorpyrifos (O, O-diethyl O-[3,5,6,-trichloro-2-pyridyl] phosphorothionate, CPF) is a common organophosphate insecticide, acaracide and miticide. It has been widely used in both agricultural and non-agricultural areas since 1965. Like other organophosphate pesticides, exposure to high doses of chlorpyrifos can lead to acute poisoning**,** by covalently inhibiting acetylcholinesterase (AChE), overstimulating the nervous system causing neuromuscular symptoms and at very high exposures initiates seizures, respiratory paralysis and death. In addition, there are human epidemiological studies demonstrating that long-term, low-level exposure to chlorpyrifos can lead to chronic neurotoxicity in the absence of cholinesterase inhibition, including deficits in cognition, memory, emotional state and syntactic reasoning [1,2].

Human pluripotent stem cell derived cell models are important in studies of the cellular metabolism of chlorpyrifos, and the molecular and cellular processes involved in its chronic neurotoxicity. Until now, no methods have been reported to quantitate chlorpyrifos and its metabolites in cells and culture media. In this paper, we have established and validated a method, with protein precipitation for cell sample preparation and liquid-liquid extraction for media sample preparation using LC-MS/MS for detection, which simultaneously quantitates CPF, CPO and TCP. After validation, this method was applied to analyze neurons treated with CPF to study the neurotoxicity. This method also facilitates investigations into the neuroprotective role of astrocytic cytochrome P450s against chlorpyrifos exposure in an astrocyte-neuron co-culture system.

Materials and Method: Chlorpyrifos (CPF, 99.5% pure), chlorpyrifos oxon (CPO, 98.5% pure), and 3,5,6- trichloro-2-pyridinol (TCP, 99% pure) were all purchased from Chem Service (West Chester, PA). The chemical structures are shown in Fig. 1. Ammonium acetate, isopropyl ether, LC-MS grade methanol, acetonitrile, water and formic acid were purchased from Sigma-Aldrich (St. Louis, MO). Human pluripotent stem cell derived NeuroNet™ pure human neurons (DIV 28 neurons) and culture media (AB2™ basal media supplemented with ANS™ neural supplement) were acquired from ArunA Biomedical (Athens, GA).

LC-MS/MSconditions

A Zorbax Eclipse XDB-C8 (2.1×150 mm, 5 µm) column coupled with a Phenomenex SecurityGuard C-8 guard column (4.0 mm×2.0 mm) was used for the separation. The column temperature was kept at 32°C. The mobile phase A was 0.025%formicacidinwaterandmobile phase B was acetonitrile. The injection volume was 15 µL. The analytes were separated using a gradient method, with a 0.3 mL/min flow rate, (time/minute, % mobile phase B): (0, 60), (2, 80), (2.01, 80), (5, 80), (6, 60), (10, 60). The autosampler injection needle was rinsed with methanol after each injection. Nitrogen was used as the desolvation gas at a flow rate of 500 L/h. The desolvation temperature was500°C and the source temperaturewas120°C.Argon was used as the collision gas, and the collision cell pressure was set at 3.5×10^{-3} mbar. Samples were analyzed by the mass spectrometer in positive ion mode for CPF and CPO, and in negative ion mode for TCP. In MS tune setting, the capillaryvoltage was 3.5 kV and the cone voltage was 28 V for the determination of CPF and CPO. The tune parameters were 4 kV and -22 V for TCP. Thecollision energy was 22 eV for CPF and 15 eV for CPO. Multiple reaction monitoring (MRM) functions were applied for detecting CPF and CPO, and the monitored ion transitions were 352→200and336→280, respectively. A selected ion recording(SIR)function form/z=198was applied for the detection of TCP.

Standards and QCs

The primary stock solutions were prepared at 0.5 mg/mL in acetonitrile for all analytes and stored in the refrigerator (+4 °C) when not in use. All dilutions were made using acetonitrile. Standard working solutions containing all three analytes were prepared fresh before use.

The concentrations for all standard and quality control (QC) working solutions are listed in Table 1. For sample preparation, 10 µL of a standard or QC working solution was spiked into either a cell pellet (1×10^6 cells) or 90 µL of blank cell culture media to produce the corresponding standard or QC samples.

Sample preparation

Media sample preparation: 1.7 mL of diisopropyl ether was added to each 100 µL of culture media. The mixture was vortexed for 10 min before centrifuged at 20000×g, 5 °Cfor 10 min. 1.5 mL of organic supernatant was collected and evaporated to complete dryness in the vacuum concentrator at 55 °C for 10 min. The sample was reconstituted with 100 μL of acetonitrile.

Cell sample preparation: 1 mLof methanol was added to the cell pellet (1×10^6 cells). The mixture was briefly vortexed and stored at -80 °Cfor 15 min. The cell lysate was centrifuged at 20000×g, 5 °Cfor 10 min, and 0.9 mL of supernatant was transferred to a glass tube. Another 1 mL of acetonitrile was added to the extract and evaporated to dryness. The residue was reconstituted with 100 μL of acetonitrile. The following procedures are the same as in media samplepreparation: reconstituted samples were sonicated, vortexed, and centrifuged at 15,000×g, 5 °Cfor 10 min. 80 μLof supernatant was transferred into an autosampler vial for analysis using LC-MS/MS.

Method validation

Selectivity, linearity, intra- and inter-day precision and accuracy, recovery, stability and dilution tests were conducted for method validation. Selectivity (n = 6) was tested by comparing the chromatograms of blank samples with those at the LLOQ. For media samples, the linearity was validated using calibration standard samples over the concentration ranges of 5-500, 0.2-20 ...and 20–2000 ng/mL for CPF, CPO, and TCP, respectively. The linearity ranges were 0.5–50, 0.02–2, and 2–200 ng/million cells for CPF, CPO, and TCP in cell samples. Calibration curves were constructed using the peak area over the concentration (ng/mL) or amount (ng/million cells), applying 1/x weighted linear regression.

The intra-day (n = 5) and inter-day (n = 15) precision and accuracy were evaluated using QC samples at the lower limit of quantification (LLOQ), and at low, middle, and high QC levels (LQC, MQC, and HQC, respectively).

Recovery (n = 3), including matrix effect, relative recovery, and absolute recovery in culture media and cell samples, was assessed by comparing peak areas of spiked samples, post-preparation spiked samples, and standard solutions at the LQC, MQC, and HQC concentrations. Autosampler stability (25 °C, 10 h) and bench-top stability (25 °C, 2 h) of the analytes were evaluated at the LQC and HQC levels.

Dilution tests (n = 5) were conducted by diluting spiked media samples to the upper limit of quantification (ULOQ) with blank media, and by diluting post-preparation spiked cell samples with post-prepared blank matrix.

Method Development

The MS parameters were optimized on the tune page with a direct infusion of the standard solution (10 μg/mL of each analyte). Positive ion mode was applied for the detection of CPF and CPO, and negative ion mode was applied for TCP, based on the intensities of the analytes under each ion mode.

In addition, CPO showed significantly higher ionization efficiency than CPF (more than 10-fold) under the same MS parameters, possibly due to the enhanced charge retention on the P=O group compared to the P=S group. Product ion mass spectra for CPF and CPO were acquired using injections of the same standard solution. The most abundant fragment ion for each analyte was selected for inclusion in the MRM function. The monitored ion transitions were m/z 352 → 200 for CPF and m/z 336 → 280 for CPO.

Fragment ions for TCP could not be detected, likely due to the stability of its aromatic ring. Therefore, a selected ion recording (SIR) function with m/z = 198 was used for TCP detection.

For sample preparation, liquid-liquid extraction was used for media samples. Various organic solvents—including isopropyl ether, dichloromethane, and ethyl acetate—were tested. All tested solvents provided similar recoveries for CPO. However, isopropyl ether provided the highest recovery for CPF (1-fold and 20% higher than ethyl acetate and dichloromethane, respectively). Dichloromethane failed to extract TCP effectively. Isopropyl ether also required the least time for evaporation. Based on these findings, isopropyl ether was selected as the extraction solvent.

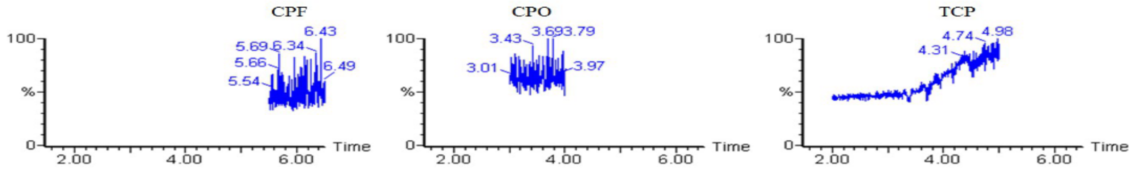
TCP is a weakly acidic compound, so a more acidic pH facilitates its neutralization in the aqueous phase and enhances its extraction into the organic phase. Recovery of TCP from cell culture media increased significantly (by 1-fold) when the media's pH was adjusted to 4 using formic acid. Therefore, cell culture media was adjusted to pH 4, and cell pellet samples were mixed with 1 µL of 10% formic acid before sample preparation.

Selectivity

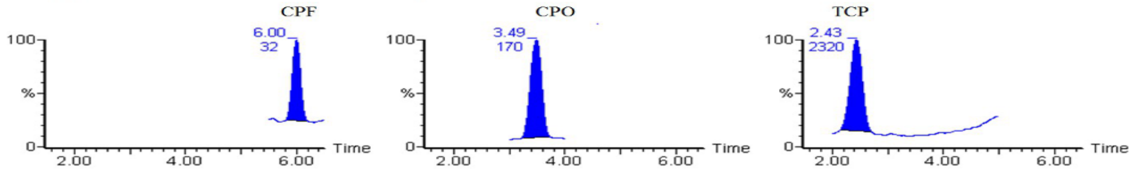
Selectivity (n = 6) was validated by analyzing blank culture media, spiked media samples (5.0, 0.2, and 20 ng/mL for CPF, CPO, and TCP, respectively), blank cell samples, and spiked cell samples (0.5, 0.02, and 2 ng/million cells for CPF, CPO, and TCP, respectively). Chromatograms of the same matrix were compared between blank and spiked samples (Fig. 2).

No significant interferences from blank matrices were observed, demonstrating that the LC-MS/MS method possesses adequate selectivity to accurately differentiate and quantify the analytes in both cells and culture media, even in the presence of matrix components.

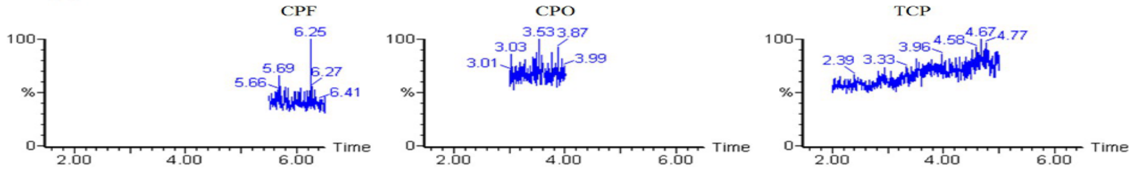
(A) Blank cell culture media



(B) Cell culture media at the LLOQ



(C) Blank cells



(D) Cells at the LLOQ

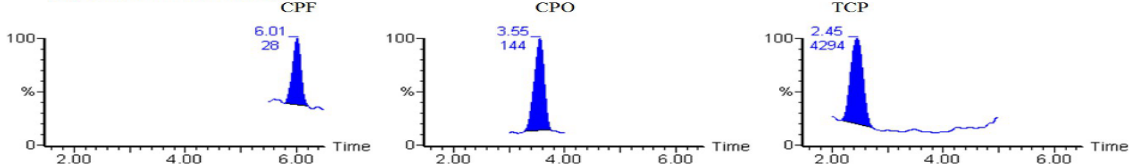


Figure: Representative chromatograms of CPF, CPO and TCP in blank cell culture media (A), cell culture media at the LLOQ (B), blank cells (C) and cells at the LLOQ (D). Retention times for analytes are shown in minutes

Linearity

The validated linear concentration ranges were: 5–500, 0.2–20, and 20–2000 ng/mL for CPF, CPO, and TCP in media samples, and 0.5–50, 0.02–2, and 2–200 ng/million cells in cell samples. Calibration curves were constructed using the peak areas of analytes, applying 1/x weighted linear regression. The slopes, intercepts, and R^2 values from the calibration curves are presented in **Table**. The method demonstrated good linearity (**$R^2 > 0.99$**) for all analytes in both matrices within the tested ranges.

Precision and Accuracy

The intra-day (n = 5) and inter-day (n = 15) precision and accuracy for all analytes were assessed using the LLQC, LQC, MQC, and HQC samples.

For cell culture media samples, the QC concentrations were as follows:

- LLOQ: 5.0 ng/mL (CPF), 0.2 ng/mL (CPO), 20.0 ng/mL (TCP)
- LQC: 15.0 ng/mL (CPF), 0.6 ng/mL (CPO), 60.0 ng/mL (TCP)
- MQC: 150.0 ng/mL (CPF), 6.0 ng/mL (CPO), 600.0 ng/mL (TCP)
- HQC: 375.0 ng/mL (CPF), 15.0 ng/mL (CPO), 1500.0 ng/mL (TCP)

For cell samples, the same absolute amounts were used but expressed in ng/million cells:

- LLOQ: 0.5 (CPF), 0.02 (CPO), 2.0 (TCP)
- LQC: 1.5 (CPF), 0.06 (CPO), 6.0 (TCP)
- MQC: 15.0 (CPF), 0.6 (CPO), 60.0 (TCP)
- HQC: 37.5 (CPF), 1.5 (CPO), 150.0 (TCP)

Relative standard deviation (RSD) was used to evaluate precision, while relative error (RE) was used to evaluate accuracy.

Table 3 presents the quantitation, RSD, and RE values for all analytes in QC media and cell samples. All RSD and RE values were within 15% (except for LLOQ, within 20%), satisfying FDA Bioanalytical Method Validation guidance.

The LLOQ was validated according to FDA requirements by confirming that RSD and RE were within 20%, and that analyte response at the LLOQ exceeded five times the blank signal.

Recovery

Absolute recovery, relative recovery, and matrix effects (n = 3) were assessed in both culture media and cell samples. Spiked samples, post-preparation spiked samples, and standard solutions at LQC, MQC, and HQC levels (in triplicate) were used.

- Absolute recovery (AR): Ratio of peak areas of spiked samples to standard solutions.
- Relative recovery (RR): Ratio of spiked samples to post-preparation spiked samples.
- Matrix effect: Ratio of post-preparation spiked samples to standard solutions.

A ratio >100% indicated matrix enhancement, while <100% indicated suppression. The extent of enhancement or suppression was calculated as the deviation from 100%.

Table summarizes the AR, RR, matrix effects, and their classification at three QC levels.

Results showed that absolute and relative recoveries were consistent for each analyte within a matrix. CPF and CPO showed weak to medium suppressive matrix effects, likely due to co-eluting matrix components interfering with ionization. In contrast, TCP showed weak to medium enhancing matrix effects, suggesting matrix components enhanced ionization in negative mode.

Stability

Autosampler stability (25 °C, 10 h) and bench-top stability (25 °C, 2 h) were validated using two sets (n = 3) of spiked media and cell samples at LQC and HQC levels.

- One set was analyzed immediately (time-zero control).
- The second set was injected after 10 h (autosampler stability).
- The third set was analyzed after 2 h at room temperature (bench-top stability).

The ratio of peak areas compared to the time-zero control was calculated. **Table 5** contains the stability results.

No significant degradation was observed, confirming the stability of analytes under sample handling and preparation conditions.

Dilution

Analyte concentrations in real samples depend on treatment dose, exposure time, metabolism, degradation, and other factors. A dilution test (n = 5) was conducted to validate accurate measurement when concentrations exceed the ULOQ.

- Culture media samples spiked at 5× ULOQ were diluted to ULOQ with blank media before sample preparation.
- Cell samples spiked at 5× ULOQ were diluted to ULOQ with post-prepared blank matrix before LC-MS analysis, due to the difficulty in transferring blank cell pellets.

Quantitative results, precision, and accuracy (n = 5) are presented in Table. All RSD and RE values were within 15%, validating the dilution procedure.

Table1. Concentrations (ng/mL) of analytes in standard and QC working solutions

Standard working solution	CPF	CPO	TCP
A	5000	200	20000
B	2500	100	10000
C	1250	50	5000
D	500	20	2000
E	250	10	1000
F	125	5	500
G	50	2	200
LLOQ	50	2	200
LQC	150	6	600
MQC	1500	60	6000
HQC	3750	150	15000

Table2. Calibration curves for CPF, CPO and TCP in cell culture media and cells (n=3). Quantitation units: cell culture media: ng/mL; cells: ng/million cells

Analyte	Cellculture media			Cells		
	Slope	Intercept	R^2	Slope	Intercept	R^2
CPF	10.1070 ±1.0505	-7.8342±14.4258	0.9957 ±0.0029	22.4304±2.0033	-4.6608±3.3870	0.9900±0.0044
CPO	332.9817 ±30.2517	-5.5474±3.4559	0.9972 ±0.0016	6585.40±834.33	-7.6712±13.2846	0.9943±0.0051
TCP	62.2681 ±0.1355	-100.9538±32.0791	0.9989 ±0.0005	1615.55±171.09	329.22±1276.01	0.9944±0.0023

Table 3. The intra-day (n = 5) and inter-day (n = 15) precision (RSD) and accuracy (RE) of the LC–MS/MS method used to quantitate CPF, CPO and TCP in culture media and cells. Units for nominal and measured levels: concentration in culture media: ng/mL, cells: ng/million cells

Matrix	Analyte	Nominal level	Intra-day			Inter-day		
			Measured level	RSD (%)	RE (%)	Measured level	RSD (%)	RE (%)
Culture media (ng/mL)	CPF	5	5.50±0.41	7.51%	10.00%	5.05±0.68	13.47%	1.00%
		15	14.95±1.21	8.09%	-0.33%	14.22±1.07	7.52%	-5.20%
		150	134.25±7.35	5.48%	-10.50%	128.45±7.35	5.72%	-14.37%
		375	349.25±42.09	12.05%	-6.87%	336.10±37.36	11.12%	-10.37%
	CPO	0.2	0.21±0.03	12.28%	5.00%	0.22±0.04	18.18%	10.00%
		0.6	0.64±0.02	3.56%	6.67%	0.58±0.08	13.79%	-3.33%
		6	5.19±0.07	1.29%	-13.50%	5.74±0.85	14.81%	-4.33%
		15	13.99±0.76	5.40%	-6.73%	15.37±1.71	11.13%	2.47%
	TCP	20	21.64±1.70	7.84%	8.20%	21.17±1.67	7.88%	5.85%
		60	59.21±7.55	12.75%	-1.32%	62.98±5.70	9.04%	4.97%
		600	596.04±71.79	12.04%	-0.66%	613.78±68.52	11.16%	2.19%
		1500	1427.20±188.04	13.18%	-4.85%	1486.53±222.11	14.94%	-0.90%
Cells (ng/million cells)	CPF	0.5	0.59±0.10	17.46%	17.00%	0.60±0.11	18.33%	20.00%
		1.5	1.58±0.20	12.58%	5.50%	1.54±0.17	11.04%	2.67%
		15	13.96±1.26	9.03%	-6.93%	13.90±1.92	13.81%	-7.33%
		37.5	36.00±2.05	5.69%	-3.99%	35.74±2.81	7.86%	-4.69%
	CPO	0.02	0.020±0.001	5.00%	0.00%	0.021±0.004	16.64%	5.88%
		0.06	0.067±0.010	14.93%	11.67%	0.070±0.009	13.23%	14.29%
		0.6	0.658±0.039	5.93%	9.67%	0.658±0.030	4.58%	5.70%
		1.5	1.668±0.112	6.71%	11.20%	1.745±0.112	6.43%	14.04%
	TCP	2	1.62±0.06	3.98%	-19.25%	1.90±0.37	19.64%	-5.00%
		6	5.13±0.40	7.77%	-14.58%	5.76±0.84	14.57%	-4.00%
		60	57.57±1.21	2.10%	-4.05%	58.72±5.12	8.73%	-2.13%
		150	143.53±20.84	14.52%	-4.31%	139.79±18.28	13.08%	-6.81%

Table 4. Absolute recovery (%AR, n=3), relative recovery (%RR, n=3) and matrix effect (%ME, n=3) of the method. Units for nominal levels: concentration in culture media: ng/mL, cells: ng/million cells

Matrix	Analyte	Nominal level	AR (%)	RR (%)	ME (%)	Type	
Culture	CPF	15	50.75±4.54	72.39±6.57	70.10%	29.90%	Suppression
media		150	52.31±2.13	81.87±3.19	63.90%	36.10%	Suppression
(ng/mL)		375	46.40±4.93	79.04±6.02	58.71%	41.29%	Suppression
	CPO	0.6	59.96±6.40	61.25±5.54	97.89%	2.11%	Suppression
		6	58.73±2.57	59.23±1.37	99.16%	0.84%	Suppression
		15	66.33±4.67	57.78±8.54	114.79%	14.79%	Enhancement
	TCP	60	45.92±1.27	44.45±1.23	103.32%	3.32%	Enhancement
		600	46.61±4.39	45.81±1.18	101.74%	1.74%	Enhancement
		1500	46.63±1.14	46.33±1.13	100.64%	0.64%	Enhancement
Cells	CPF	1.5	59.29 ±11.44	73.17 ±14.11	81.03%	18.97%	Suppression
(ng/million		15	64.39 ±7.27	72.56 ±11.58	88.74%	11.26%	Suppression
cells)		37.5	61.77 ±6.51	77.82 ±10.17	79.38%	20.62%	Suppression
	CPO	0.06	55.32 ±8.70	79.63 ±12.84	69.47%	30.53%	Suppression
		0.6	55.54 ±3.91	75.12 ±5.29	73.94%	26.06%	Suppression
		1.5	60.64 ±4.47	77.94 ±5.75	77.80%	22.20%	Suppression
	TCP	6	103.64 ±8.27	87.28 ±7.71	118.74%	18.74%	Enhancement
		60	110.81 ±14.59	92.23 ±12.14	120.14%	20.14%	Enhancement
		150	111.23 ±14.31	85.25 ±10.32	130.48%	30.48%	Enhancement

Table 5. Autosampler stability (n = 3) and bench-top stability (n = 3) of CPF, CPO and TCP at the LQC and HQC in culture media and cells. Stabilities are shown as percentages of relative concentration when compared to the time zero control (mean ± SD). Units for nominal levels: concentration in culture media: ng/mL, cells: ng/million cells

Matrix	Analyte	Nominal level	Autosampler stability (%)	Bench-top stability (%)
Culture	CPF	15	91.23 ±2.44	101.60 ±8.29
media		375	97.19 ±3.07	96.25 ±1.98
(ng/mL)	CPO	0.6	98.52 ±5.77	96.93 ±1.84
		15	92.53 ±2.11	94.01 ±0.62
	TCP	60	97.77 ±0.66	89.32 ±2.95
		1500	99.92 ±3.47	97.91 ±3.24
Cells	CPF	1.5	96.01 ±5.88	87.76 ±1.22
(ng/million		37.5	95.24 ±2.77	95.75 ±1.30
cells)	CPO	0.06	96.71 ±7.11	98.49 ±0.77
		1.5	98.54 ±2.44	97.76 ±1.21
	TCP	6	100.17 ±5.61	97.92 ±2.24
		150	97.99 ±2.99	101.14 ±3.01

Table 6. Precision (RSD) and accuracy (RE) of spiked samples (n = 5) at 5-fold the ULOQ in culture media and cells diluted to the ULOQ

Matrix	Analyte	Nominal level	Measured level	RSD (%)	RE (%)
Culture	CPF	2500	2426.07 ±140.81	5.80%	-2.96%
media	CPO	100	95.49 ±7.00	7.33%	-4.51%
(ng/mL)	TCP	10000	9951.33 ±1067.47	10.73%	-0.49%
Cells	CPF	250	246.33 ±16.50	6.70%	-1.47%
(ng/million	CPO	10	10.27 ±0.75	7.31%	2.70%
cells)	TCP	1000	949.33 ±31.00	3.27%	-5.07%

Table 7. Quantitation of CPF, CPO and TCP in culture media and cells, obtained from culture media incubated with 10 µM CPF for 48 h (Blank media + CPF) and DIV 28 neurons treated with 10 µM CPF under the same conditions (Neurons + CPF) and. N/A: not applicable

Treatments	Analyte	Conc.in culturemedia(ng/mL)	Levelsincells(ng/millioncells)
Blankmedia+CPF	CPF	140.07 ±15.76	N/A
	CPO	0.78 ±0.01	N/A
	TCP	84.23 ±10.87	N/A
Neurons+CPF	CPF	105.80 ±13.10	725.76 ±180.17
	CPO	0.18 ±0.03	0.19 ±0.03
	TCP	74.72 ±9.00	2.52 ±0.15

References

1. S.M.Ross,I.C.McManus,V.Harrison,O. Mason,Crit.Rev.Toxicol.43(2013)21-44.
2. D.S.Rohlman,W.K.Anger,P.J.Lein,Neurotoxicology32(2011)268-276.
3. C.M. Hernandez, W.D. Beck, S.X. Naughton, I. Poddar, B.L. Adam, N.

Yanasak, C. Middleton,A. V Terry, Neurotoxicology 47 (2015) 17-26.

4. A.VTerry,D.A. Gearhart,W.D.Beck, J.N.Truan,M.L.Middlemore, L.N.Williamson,M.G. Bartlett, M.A. Prendergast, D.W. Sickles, J.J. Buccafusco, J. Pharmacol. Exp. Ther. 322 (2007) 1117-1128.
5. A.V Terry, J.D. Stone, J.J. Buccafusco, D.W. Sickles, A. Sood, M.A. Prendergast, J. Pharmacol. Exp. Ther. 305 (2003) 375-384.
6. R.J.Foxenberg,B.P.McGarrigle,J.B.Knaak,P.J.Kostyniak,J.R.Olson,DrugMetab.Dispos. 35 (2007)189-193.
7. F.M. Buratti, M.T. Volpe, A. Meneguz, L. Vittozzi, E. Testai, Toxicol. Appl. Pharmacol. 186 (2003) 143-154.
8. F.Eyer,D.M.Roberts,N.A.Buckley,M.Eddleston,H.Thiermann,F.Worek,P.Eyer, Biochem. Pharmacol. 78 (2009) 531-537.
9. A.L.Crane,K.Klein,U.M.Zanger,J.R.Olson,Toxicology293(2012)115-122.
10. S.Das,T.K.Adhya,J.Environ.Manage.152 (2015)36-42.
11. J.W.Albers,S.Berent,D.H.Garabrant,B.Giordani,S.J.Schweitzer,R.P.Garrison,R.J. Richardson, J. Occup. Environ. Med. 46 (2004) 367-378.
12. X.K.Yang,M.G.Bartlett,RapidCommun. MassSpectrom.30(2016)652-664.
13. L.N. Williamson, M.G. Bartlett, A. V Terry, J. Liq. Chromatogr. Relat. Technol. 30 (2007) 273-285.
14. J.VSancho,O.J.Pozo,F.Hernandez,Rapid Commun.MassSpectrom.14(2000)1485-1490.
15. W.Bicker,M.Lammerhofer,W.Lindner,J. Chromatogr.B-AnalyticalTechnol.Biomed.Life Sci. 822 (2005) 160-169.
16. L.N.Williamson,A.VTerry,M.G.Bartlett, RapidCommun.MassSpectrom.20(2006) 2689-2695.
17. R.Heilmair,F.Eyer,P.Eyer,Toxicol.Lett.181(2008)19-28.
18. S.L.Miksys,R.F.Tyndale,J.PsychiatryNeurosci.27(2002)406-415.