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Leaching behavior of MCPA pesticide in Brewed tea

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Abstract

The objective of this paper was to investigate the leaching behavior of MCPA residues during the preparation of tea infusion. The analysis was carried out by the modified QuEChERS extraction method using LCMS/MS. Method validation was also performed for the quantification of MCPA at different fortification levels: 5, 10 and 25 µg/Kg and 0.5, 1 and 2 µg/Kg in Tea and Brewed tea respectively. Calculated recovery percentage was within the acceptable range of 80% -120%. In Tea, the LOD and LOQ was 2.5 µg/Kg and 5.0 µg/Kg, whereas in brewed tea, it was 0.25 µg/Kg and LOQ 0.5 µg/Kg respectively. To compensate matrix effects for accurate quantification, representative matrix-spiked calibration curves were applied. The results showed that the release of MCPA decreased in boiled water with increase of brewing time, and the highest transfer was observed during the first 3 minutes. The transfer rate of MCPA to the brewed tea appeared to be dependent on its water solubilities and partition coefficient (Kow).

Keywords: MCPA, Method Validations, QuEChERS, brewed tea, infusion, residues

1. Introduction

Tea is the most popular nonalcoholic beverage in the world. Many studies and researches on Tea found that tea is a treasured elixir and is consumed for its alluring flavors and health benefits. Due to health awareness among the consumers, there is increase in demands for these products and subsequently tea cultivation. In recent years there been rapid expansion in tea crops involving both black and green tea.

As like other agricultural commodities, tea crops are also prone to pest's attacks. In recent years, public awareness and concern over the presence of pesticide residues in food products including tea and its toxicity has increased. Tea represents a significant potential source of human exposure to pesticide residues by virtue of high application of pesticides to tea crop coupled with the average intake of six grams of dried (made) tea per day

per individual [1, 2]. Each tea growing areas has its own idiosyncratic pests and diseases, though several of them might have been recorded from more than one region [3]. To minimize plant losses, chemical control of pests and plant diseases has been introduced worldwide, but the continued widespread overuse of pesticides has created serious acute health problems, along with local and global environmental issues, especially in the developing countries [4, 5]. In addition, conjugation with endogenous macromolecules may result in the formation of non-extractable residues. [6]. One of the major disadvantages of pesticide use is that residues may remain on tea and can be transferred to the infusion (brew) at amounts higher than the MRLs and may pose health hazards [7].

In addition, pesticide levels decrease due to the natural factors, including rainfall, dew, volatilization, airflow, photolysis, biodegradation, moisture, pH and growth dilution [8] and the pre-harvest interval between the last application and harvesting [9]. It has also been found that during the different drying stage of tea manufacturing, the pesticide residues are substantially reduced. The tea industry in general uses pesticides which are cleared by the European Union for maximum residue limit (MRL) in tea. Generally, a total of three to four rounds of acaricides and one or two rounds of insecticides are sprayed in tea gardens for controlling mites and insect pests of tea in areas, depending on intensity of infestation. Photolabile compounds undergo degradation due to sun light [10; 11]. For several pesticides, the European Union (EU) residue definitions for monitoring established within the Regulation on maximum residue levels (MRLs) of pesticides in or on food and feed of plant and animal origin [12] include esters and conjugates of acids. These conjugation in plants may occur with natural compounds present in the plants (e.g., sugars, polysaccharides, amino acids, proteins, and plant cuticles containing epoxy groups). Unlike green or black tea, herbal tea does not require further processing, such as fermentation [13], and can, thus, contain higher levels of pesticide residues, with additional health risks for the consumers.

However, there is also evidence that pesticides can be transferred from the tea matrix to the infusion during preparation [14]. Tea, in particular green tea, is infused repeatedly for several minutes until no taste or colour is produced. The hot water extract of tea (tea brew) is consumed and not the tea as a whole. Therefore, tea brew could be a possible source of contamination. Actual exposure depends on the quantity of the residues leached into the tea brew and not the mere presence of residues in/on the tea [15]. Recent study shows that pesticide residues were likely transferred from tea leaves to brewed tea during the brewing process, and may therefore pose a risk to consumers. The work done so far on pesticide residue aspects of tea is quite scarce, although, some published papers described the loss of organophosphates, pyrethroids, and γ -HCH and DDT residues in black or green tea [16, 17]. Many papers described the determination of Organochlorine residues in black tea [18] and in linden and camomile [19, 20], but not in their infusions. However, Nagayama [21] observed 2 to 52% transfers of residues of organophosphates into brewed tea.

To fulfil the world's growing demand, application of herbicide is necessary. Herbicides are a specific group of plant protection products (PPP) used to treat broad leaved weeds and other associated weeds which may reduce crop productivity. The phenoxyacetic acid herbicides are one of the most commonly used groups of PPPs because of their low cost, effectiveness and good water solubility [22]. MCPA (4-Chloro-o-tolyloxyacetic acid), **Figure 1** is a powerful, selective, widely used phenoxy herbicide. MCPA is used as an herbicide, generally as its salt or esterified forms has been extensively used in agriculture to control broad-leaf weeds as a growth regulator.

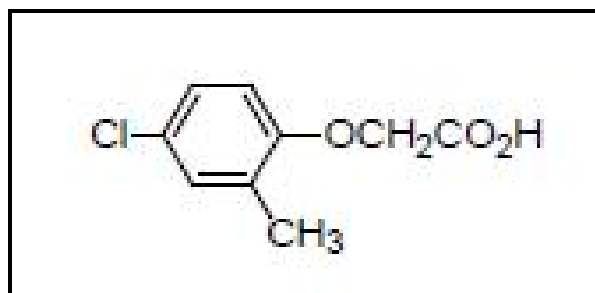


Figure 1: Structure of MCPA

Analytical methods for pesticide residues should be able to measure all components including its conjugated residues if any. The extraction method must include appropriate techniques for releasing the conjugated moiety. The present study aims, to study the transfer of MCPA pesticide from tea leaves to its brew with varying brewing time in comparison to the analysis of MCPA residues in tea leaves. Also with the objective to validate a sensitive modified method for extraction of MCPA and its conjugates using LCMS/MS technique.

2. Materials and Methods

2.1 Chemicals and reagents

MCPA (4-chloro-o-toloxycetic acid) of 98.7% purity was obtained from Chem Service India Ltd. All the chemicals used were of analytical grade (AR) or high performance liquid chromatography (HPLC) grade. Glasswares of Borosil make and Milli-Q water was used during the study. Black tea leaves were procured from the local market.

2.2. Preparation of standard solutions

Approximately 10mg MCPA was accurately weighed in a 10ml volumetric flask. To this 5ml of Methanol was added and ultrasonicated to ensure complete dissolution. The volume was made upto the mark with Methanol. This gave a standard stock solution having concentration of approximately 1000 mg/Kg. From this stock solution, working standards of 5, 10, 25, 50 and

100 µg/Kg concentration and 0.5, 1, 2, 5 and 10 µg/Kg of MCPA were prepared by serial dilution for residual analysis in tea leaves and its brew respectively.

2.3. Extraction and Clean-up

For residual analysis in tea leaves: About 2.5 g (± 0.25 g) of the Tea leaves was taken in a 50ml capacity centrifuge tube and 10 ml of water (HPLC grade) was added for homogenization. It followed with the addition of 10 ml acetonitrile and hand shaken for 1 min and added 0.7ml 5N NaOH, tubes were capped and vortexed for 1-2min and then incubated at 40°C for 30 min in water bath with shaker. Cooled and added 0.4ml 5M H₂SO₄. To this directly added 4g anhydrous MgSO₄, 1g NaCl, 1g Na₃Citrate and 0.5g Na₂H Citrate sesquihydrate. Tubes were sealed tightly and shaken to ensure complete mixing, centrifuged for 10 minutes at 10,000 rpm in a centrifuge machine. Aliquot of about 6ml from the supernatant layer was transferred into 15ml capacity centrifuge tubes and added 150 mg of C18, 900 mg of anhydrous MgSO₄ and 45 mg GCB. After vortexing, it was centrifuged at 10,000rpm for 5 min. 2ml of the supernatant was completely dried and made-up with 2ml of 1:9 mixture of ACN:Water. After filtering through 0.45µm filter membrane, it was injected onto LCMS/MS and the identified peaks was calculated against the response factor of the respective standard solutions of MCPA (Salts, Free acids, Esters & Conjugates).

For residual analysis in brewed tea: Brewed tea was prepared with fortified tea leaves. About 2.5 g (\pm 0.25 g) of the fortified Tea leaves was immersed in 100ml of boiling water separately in three different beaker and after brewed for 3, 5 and 10 minutes respectively, the water extract was filtered, cooled and transferred to a conical flask. 5ml of the each water extract was taken separately in 50ml capacity centrifuge tubes and added 10 ml of HPLC grade water to it followed by addition of 10ml acetonitrile and 0.7ml 5N NaOH, vortex for 1-2min and then incubated at 40°C for 30min in water bath with shaker. After cooling, added 0.4ml 5M H₂SO₄ and the same extraction procedure was followed as in tea leaves by adding QuEChERS salts and final injection onto LCMS/MS.

2.4. Method Validation

The method has been validated for determination of low level of MCPA in Tea leaves and brewed tea. The validation involved the analytical curves linearity, limits of detection (LOD) & quantification (LOQ) and recoveries. For the determination of the LOD and LOQ, different concentrations of the standard solution were injected. The analytical curves were constructed by injecting working standard solutions of 5, 10, 25, 50 and 100 $\mu\text{g/Kg}$ and 0.5, 1, 2, 5 & 10 $\mu\text{g/Kg}$ concentration prepared in the matrix extract for quantitative analysis of MCPA in tea leaves and its brew respectively.

In order to establish the reliability of the analytical methods including the extraction and clean-up steps, **Recovery studies** were also carried. Recovery was performed by spiking matrix blank at the three different fortification levels: 5, 10 and 25 $\mu\text{g/Kg}$ and 0.5, 1 and 2 $\mu\text{g/Kg}$ with three replicates at each level in Tea and Brewed tea respectively. All spiked samples were left to stand for an appropriate period of time to allow

the spiking solutions to penetrate the matrix. The fortified samples were extracted and cleaned up following the procedure given in the preceding section.

2.5. Instrumentation analysis of MCPA

Liquid chromatography was achieved using an Agilent 6460 system. The system consisted of a binary pump, a vacuum solvent degasser unit, a column oven, and a temperature-controlled automated liquid sampler. The separation was performed using C18 column, 150 mm \times 4.6 mm internal diameter, and 3.5 μm particle sizes. The flow rate was 0.5 mL/min (gradient). The injection volume was 10 μL . Mobile phase A was Water/ Methanol with 5mM Ammonium formate (80/20 v/v), and mobile phase B was Methanol/ Water with 5mM Ammonium formate (90/10 v/v). The total run time was 25 min. Electrospray ionization (ESI) in negative mode for analyte was used.

3. Results and Discussion

The method validation was done by evaluating Specificity, linearity, Limit of Detection (LOD), Limit of Quantitation (LOQ), Recovery. The specificity criterion tries to demonstrate that the result of the method is not affected by the presence of interferences and for this the chromatograms of the spiked samples showed no such interference. The linearity of an analytical method is its ability within a definite range to obtain results directly proportional to the concentrations (quantities) of an analyte in the sample. Matrix match linearity was constructed at five point concentration over a range of 5 to 100 $\mu\text{g/Kg}$ for tea and 0.5 to 10 $\mu\text{g/Kg}$ for brew. The correlation coefficient (R^2) value was found to be higher than 0.99 and the calibration curves were linear within the range (**Figure 2**).

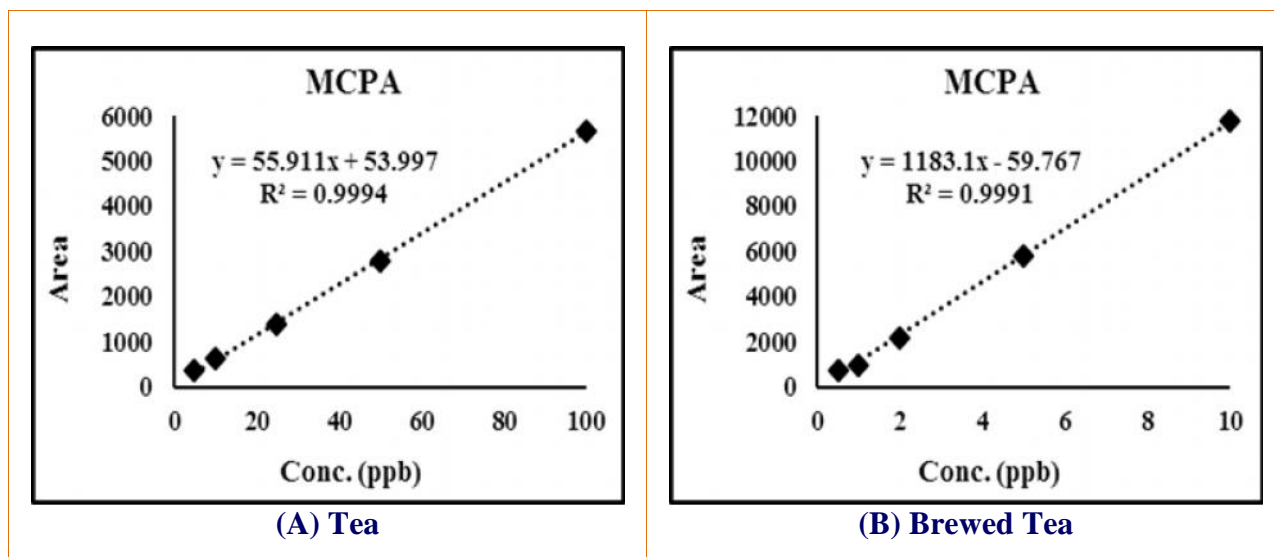


Figure 2: Linearity graph of MCPA

The LOD and LOQ were calculated by instrumental and statistical methods. LOD was determined as the lowest amount to detect and LOQ as the lowest amount to quantify, by the detector. The LOD and LOQ values suggest that method is also sufficiently reliable for detecting the expected concentrations of the examined residues during processing. The LOD and LOQ were determined based on Linearity. For LOD a value of signal-to-noise ratio equal to or greater than three ($S/N \geq 3$) was chosen. For LOQ a value of signal-to-noise ratio equal to or greater than ten ($S/N \geq 10$) was chosen. In Tea, the LOD was 2.5

$\mu\text{g/Kg}$ and LOQ 5.0 $\mu\text{g/Kg}$, whereas in brew, LOD was 0.25 $\mu\text{g/Kg}$ and LOQ 0.5 $\mu\text{g/Kg}$.

The recovery of an analyte is the extraction efficiency of an analytical process and is reported as a percentage of the known amount of an analyte carried through the sample extraction and processing steps of the method. The recovery was evaluated at three concentration levels 5, 10 and 25 $\mu\text{g/Kg}$ in tea and 0.5, 1 and 2 $\mu\text{g/Kg}$ in brewed tea. The relative standard deviations (RSDs) for all spiked levels were lower than 20%. The recovery data is as shown in **Table 1**.

Table 1: Data for Analytical Recoveries of MCPA in Tea leaves & Infusion

Matrix	Fortification Level ($\mu\text{g/Kg}$)	Mean Recovery ($\mu\text{g/Kg}$)	% Recovery \pm SD
Tea leaves	5	5.88	117.6 \pm 3.3
	10	10.20	102.0 \pm 1.6
	25	25.92	103.7 \pm 1.8
Infusion	0.5	0.54	107.3 \pm 4.4
	1	1.03	102.6 \pm 1.5
	2	2.00	100.1 \pm 1.8

The MRLs of MCPA is fixed in teas but not in infusions/ brew. In brewed tea, the tea extract in hot water is consumed. Hence, tea brew could be a possible source of contamination. In the present study the effect of brewing time and different concentration was investigated. Generally, the transfer rate depends on the two major factor i.e. water solubility and partition coefficient [23]. There is a relationship between water solubility

and the partition coefficient (Kow). Pesticides with large Kow values are strongly bound to plant tissue matrices and do not move with circulating water and, in turn, are not leached into tea water extracts. Brew time and temperature can also contribute to the transfer rate [24, 25]. The water solubility of MCPA is 26.2 g/L and also has low Kow value of 0.28-0.59.

Table 2: Residue and contaminants levels in tea infusions with respect to infusion time and fortified concentration.

Infusion Time (minutes)	Fortification concentration (~g/Kg)				
	0.5	1	2	5	10
3	0.48	0.89	1.87	5.02	9.37
5	0.35	0.71	1.42	3.85	7.23
10	0.28	0.59	1.10	3.02	5.53

The fortified tea at five different concentrations were subjected to different brewing intervals (3, 5 and 10 minutes). Going from the first to the third brewed tea, the transferred proportions (relative to the initial concentration) of MCPA decreased from tea leaves into brew gradually with increasing brewing intervals. The results as given in **Table 2** showed that the release rates of the MCPA in boiled water decreased with increasing brewing time, and the highest transfer was observed during the first 3 minutes. The total transfer rates measured for the 5 and 10 minutes were approximately 70% lower than that of the first brewing time irrespective of the concentration of MCPA subjected for infusion. The transfer rate of MCPA under this study primarily appeared to be dependent on their water solubilities and partition coefficient and the data obtained also satisfactorily substantiate a relationship between the transfer rate to brewed tea considering the two major factors i.e., water solubility and partition coefficient (Kow).

4. Conclusion

A modified method for extraction of MCPA (Salts, Free acids, Esters & Conjugates) and its quantification using LCMS/MS technique has been developed successfully. The recovery tests highlighted the good accuracy and repeatability of the method, and the method was within the acceptable range for residue determinations. In the present study, it was also found that a major amount of MCPA is leached into the tea brew within 3 minutes of infusion indicating that the extent of leaching depends on water solubility and partition coefficient of the pesticide and also depends on the brewing time.

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