Methodology Development for Routine Estimation of Chlorpropham in Commercial Potato Stores

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Abstract

Khan W.A., Duncan H.J., Baloch A.K. McGowan G. (2012): **Methodology development for routine estimation of chlorpropham in commercial potato stores**. Czech J. Food Sci., **30**: 67–73.

Chlorpropham is employed worldwide as an anti-sprout chemical to the harvested potato tubers during storage. A simple and precise analytical technique is developed for routine estimation of the sprout suppressant from a large number of potato samples supplied from commercial stores demanding quick analysis. Chlorpropham is extracted completely from potato tubers by intelligent reflux extraction followed by quantification using GC-FID (Gas Chromatography-Flame Ionization Detector) equipment. In this article, the performance of the technique is compared with the lengthy extraction/cleanup process, and the results are validated as per one-way analysis of variance. The recommended technique is found to offer rapid, accurate, and reliable analytical results with ease in handling a large number of samples constituting a wide range of residual chlorpropham levels often found in a complex structural commercial potato stores.

Keywords: chlorpropham; sprout suppressant; anti-sprout; soxhlet extraction; potato

Chlorpropham is an important anti-sprout chemical for potato tubers employed for years by commercial stores in Europe and USA, and in practice worldwide. It is mainly applied in the form of aerosol fog with a dose of 20 mg/t, three to five times a season. With the constructional complexities of the commercial potato stores, the expected uneven distribution of chlorpropham brings about a variable damage to the produce in different parts within the store causing great economic losses to potato dealers as a result of sprouting in those portions which received inappropriately low chlorpropham levels. In order to realise a higher yield of the produce, a dose higher than the recommended one is usually added to the stores to increase effectiveness of the chemical. In recent years, there are growing worries about the levels of chemical residues, particularly CIPC, in potato tubers. Most of the concerns are related to their potential adverse impacts on human health, as CIPC may potentially damage liver, kidney, spleen, and erythrocytes (Nakagawa *et al.* 2004); and their environmental impacts on ozone depletion (Kerstholt *et al.* 1997). Another factor with chlorpropham has been the occurrence of contamination of seed tubers, the growth and development of which are obviously affected by the chemical application. Under the situation, the determination of pesticide residues in agricultural products and food is of great public and regulatory concerns. It is also well known that the results of the pesticides measurement show a strong dependence on the extraction method used and the following sample clean-up methods employed for the analysis. Since there is an extensive growth in the numbers and sizes of commercial stores with a fast exchange

of potatoes, the systems demand a rapid and on line method for quantification of chlorpropham within an acceptable level of accuracy. More over, the adopted methodology should be capable of handling a large number of samples intended for analysis in the shortest time possible and applicable as a routine analytical technique by a laboratory having minimal resources. Since the adoption of an estimation method with specific aims is a vital step, the choice from the existing methods cannot be made unless the overall problem is defined. The basic procedure involved in the determination of chlorpropham in biological material includes four fundamental steps like the sample preparation, extraction, purification (clean up), and estimation of the compound at as low a level of the chemical as reported to be effective against the plant growth (< 0.05 mg/g tuber weight). Since chlorpropham is widely used as herbicide worldwide, many techniques have been advanced and reviewed (Ritchie *et al.* 1983; Beernert & Hucorne 1991; Lewis *et al*. 1996; Ascenzo *et al.* 1998). In most early cases, hydrolysis of chlorpropham was followed by column chromatography as a cleanup procedure and subsequently by quantification by means of the methods mostly based on the measurement of the developed colour. Later on, much attention was focused on the development of the extraction procedure for active components from potato tubers and other biological materials by means of multi solvent system, and on the quantification of the pesticide residues with the help of relatively refined devices of chromatography including GLC (Corsini *et al*. 1978; Batora *et al.* 1981). This eventually lead to evolving multi-residue methods mostly comprising extensive extraction through QuEChERS-like (quick, easy, cheap, effective, rugged, and safe) and accelerated solvent extraction (ASE) procedures followed by purification and concentration of the extracts by means of HPLC, and subsequently quantification via complicated devices comprising mostly GC-Mass Spectroscopy (LC-ESI-MS/MS) fitted with a Turbo Ion Spray ionisation source and/or other devices (Ascenzo *et al.* 1998; Chen *et al*. 2010; Kadamne & Proctor 2010). These techniques produce high extraction and quantification profiles and offer many advantages including cost effectiveness. However, there are many factors (also based on local demands), which can play role in the final evaluation, thus a compromise has to be made between these factors in order to develop an optimised method for the routine use since the highly developed devices with complex and laborious extraction processes employing exceedingly sophisticated instrumentations could gain least acceptance to emerge as routine analytical technique.

The present study is aimed at supplementins the existing method devised in this department and still in use (Boyd 1988). Under this technique, chlorpropham is extracted by repeated blending of potato tissues with solvents and the extract after purification/concentration, using phase separation and column chromatography, is quantified for chlorpropham with GC-FID. The process of extraction/purification of the reference technique is replaced with a modified reflux extraction procedure often used by pesticide industry (Heras & Sanchez 1982). It is further aimed at the overall refining of the methodology so as to make it applicable for routine analysis of a large number of samples from the commercial potato stores having chlorpropham levels within the Codex limit. The workability of the two methods is compared and the silent features of the developed technique in terms of accuracy and reproducibility of the results are discussed in the following sections.

MATERIAL AND METHODS

Sample preparation. The potatoes used as blank samples were purchased from a local supermarket. Two to three kg of undamaged and disease free potato were taken and washed with cold tap running water to remove dust/soil from the tuber surface. Each individual potato tuber was cut lengthwise into two halves which were cut again into two lengthways pieces each and then sliced into small cubes (2–4 cm) using a stainless steel kitchen knife and a chopping board. After mixing thoroughly, the pieces were homogenised (top drive macerator; Thompson and Mercer, Corydon, UK) to get a uniform sample which was then subdivided into two equal lots. The samples were put in pre-labelled polyethylene bags and stored immediately in a freezer (–18°C) for further experimental study.

Extraction of chlorpropham from potatoes. For extraction with solvent blending method, five separate batches of 50 mg each were taken from the sub-sample and spiked with 1ml of 100 µg/ml standard solution of chlorpropham. The sample was homogenised by mincing in electric grinder

for 1 min with 150 ml hexane in the presence of 80 g anhydrous sodium sulphate. The content along with hexane washings were taken into an aluminium bottle, which was shaken on a wrist shaker for at least 30 min, and then left for 24 h in order to extract chlorpropham from the potato tissues. The extract was then passed through filter paper on a Buchner assembly containing anhydrous sodium sulphate to get a clear solution. The residue was washed thrice with 50 ml hexane, and the combined filtrate was concentrated to 1 ml on a rotary evaporator keeping the temperature below 40°C to prevent chlorpropham losses. The extract was then applied on to an alumina column for the sample cleanup, and eluted with hexane at a maximum flow rate of 1.0 ml/minutes. The extract was concentrated to 2 ml in hexane and chlorpropham was determined by GC/FID technique.

The procedure of reflux extraction method was a modification of the technique commonly employed by the crisp industry (Lewis *et al*. 1996). Further modification was made in the reflux setup by changing the model of the reflux apparatus in such way as to allow the solvent vapours to pass round the thimble containing the sample for more rapid extraction. A subsample of 50 g was taken into the thimble of the reflux unit and spiked with 1 ml of 100 µg/ml standard solution. Five replications were run simultaneously. Chlorpropham was extracted from the samples in a reflux unit for two hours with *n*-hexane (40°C, 150 ml) as a solvent. The extract was then passed through the Buchner assembly containing Whatman No. 1 filter paper with an overspread layer of anhydrous sodium sulphate (100 g). The material was properly washed with *n*-hexane (50 ml) three times, and the total filtrate was concentrated using rotary vacuum evaporator at a reduced temperature. The potato samples were also taken from a conventional potato box store maintained at 8–10°C, and extracted similarly as per required procedure described for each case.

Estimation of chlorpropham. The concentrations of chlorpropham were determined by gas chromatography (GC) instrument with flame ionization detectors (Pye Unicam Ltd., Cambridge, UK). The chromatogram was developed under specified analytical conditions employing a glass column (2 mm *i.d.*), stationary phase of 3% OV17 (80–100 mesh) on WHP, F.I.D detectors at 230°C temperature, injector temperature of 220°C, oven temperature of 174°C, nitrogen 25 cm^3/min as a carrier gas, with the sample volume of 0.5 µl. The chlorpropham values of the peaks in the GC were finally compared with the standard values (0.01–10.0 mg/kg range) used for standardising the GC equipment. In order to assess the accuracy in the injection technique, and to check the output and performance of the GC under the prescribed conditions, the standard solutions of chlorpropham in hexane were kept running through the system for a few days by injecting the samples at the start, in the middle, and at the end of the daily work. In order to compare the recoveries, blank samples were prepared after spiking with the standard solution of 100 µg/ml of chlorpropham in hexane, and the efficiencies of the two methods were compared on the basis of minimum detectable level of chlorpropham in potatoes, and for this purpose blank potato samples were run side by side. The samples were spiked with different known concentrations of the standard solution (0.01–10.0 mg/kg range), extracted, and concentrated to 2 ml volume. 0.5 µl of the spiked samples were then injected into GC-FID and the chromatogram was developed. Glass-distilled grade of *n*-hexane supplied by Rathburn Chemical Ltd. (Walkerburn, Scotland) and Analytic Reagent grade of anhydrous sodium sulphate, Hopkin and Williams, were used in these studies. All the results were compared statistically by one-way analysis of variance using the computer Minitab 10.1 version.

RESULTS AND DISCUSSION

The recovery results obtained after the extraction of chlorpropham by the blending as well as by the reflux method are presented in Table 1. The recoveries from five replications ranged from 77.17% to 88.27% and from 93.65% to 97.05% with

Table 1. Recovery of chlorpropham (in %) from spiked potato samples with two extracting methods

Replication	Method of extraction	
	blending	reflux
1	85.96	96.00
2	88.27	97.05
3	82.27	94.42
4	80.16	94.60
5	77.17	93.65
Average recovery	82.77	95.14
Standard deviation	4.44	1.36

the average recovery of 82.77 ± 4.44% and 95.14 ± 1.36% employing the blending and reflux methods, respectively. In the case of the reflux method, the recovery was about 12% higher with more than 3 times greater precision in the determinants. Although the averaged recovered values from both of the methods were close enough and statistically there was no significant difference, the recovered chlorpropham amounts, however, were higher with better precision using the reflux extraction technique. Whereas the lower recovery percentage in the blending method might be due to incomplete extraction of chlorpropham from the sample tissues in addition to the likely losses due to the involvement of more transferring and separation/cleanup steps yielding reduced amount of chlorpropham with low precision as a consequence. Hence the process of blending with solvent extraction requires extra care and sharp adherence to each step so as to maximise the recovery of chlorpropham (Boyd 1988). Thus it was decided to follow the reflux extraction method for further analysis, and to optimise the technique.

Improvement using reflux extraction

During the reflux operation, it was experienced that the hexane extract became slightly separated into hexane and aqueous phases due to the content of moisture in the fresh sample that tended to block the thimble stopping the process eventually. This required shaking time and smooth refluxing again. Since the process was disrupted several times, the precision of the results suffered from relatively higher standard deviation values being obtained in the replications. In order to continue the refluxing process to completion, frequent cleaning up of the thimble pores became necessary. Therefore to avoid emulsification, a few further improvements in the methodology were required. Vliet and Hertog (1966) suggested either to allow soaking of tuber pieces for 3–10 days in organic solvent, or to homogenise the tissue with sufficient anhydrous sodium sulphate for rapid filtration. The latter procedure was tried as it seemed to be more plausible in the present case. Therefore, a trial was set up to introduce anhydrous sodium sulphate into the thimble before adding the sample. In order to evaluate the effect of anhydrous sodium sulphate added, a frozen sample that had been treated at the store with the sprout suppressant chlorpropham was taken, and after chopping and homogenising it was divided into two lots. With one set of 5 replications, the sample (50 mg) was taken into the thimble with added 30 g anhydrous sodium sulphate while keeping the other series without salt. The extracts that had not been dried in the thimble with anhydrous sodium sulphate were subsequently filtered through a pad of anhydrous sodium sulphate on a Buchner funnel. All of the moist extracts were then evaporated by rotary evaporation under vacuum and made up to 2 ml, and 0.5 µl sample extract was then injected into the GC and the amount of chlorpropham was estimated (Table 2). Since the blending method requires a lot of solvent for the extraction of large sample sizes (roughly 250 mg), it was not always convenient. The sample with anhydrous sodium sulphate added to the thimble before placing the sample gave an average value of 2.91 ± 0.01 mg/kg chlorpropham. The other sample extracted using the same procedure but without added anhydrous sodium sulphate gave a lower average value of 2.85 ± 0.12 mg/kg (Table 2). Although the amounts of chlorpropham from both the series were close, the sample with added anhydrous sodium sulphate in the thimble had a much lower standard deviation value. Besides showing no interference from the added anhydrous sodium sulphate, the recoveries of chlorpropham were more reproducible and the results were precise enough. Moreover, the procedure removed the stage of repeated cleanup and hence shortened the time taken for the sample extraction.

Table 2. Effect of anhydrous sodium sulphate addition on performance of the reflux extraction methodology

Replication	Anhydrous sodium sulphate (mg/kg)	
	not added	added
1	3.01	2.92
2	2.70	2.92
3	2.90	2.90
4	2.80	2.91
5	2.86	2.92
Average recovery	2.85	2.91
Standard deviation	0.12	0.01

Effect of sample size

The next trial was thus conducted to check the efficiency of the technique for a wide range of the sample sizes. To determine the optimum weight

Table 3. Effect of size of stored treated samples on estimation of chlorpropham residue

Replication	Sample size (g)				
	5	10	20	30	40
1	3.79	3.04	3.10	3.29	3.16
2	2.16	3.27	2.97	3.19	2.90
3	3.28	2.98	3.24	3.25	3.30
4	2.41	2.81	3.21	3.24	2.11
5	3.61	3.24	3.01	3.27	3.20
Mean (mg/kg)	3.05	3.07	3.10	3.25	2.93
Standard deviation	0.73	0.19	0.12	0.04	0.49

of the potato sample for the estimation, a set of five replicates of different weights (5–40 mg) from homogenised chopped potato samples were taken, and each was spiked with the standard solution of 100 µg/ml (chlorpropham in hexane) and extracted by reflux as above. Anhydrous sodium sulphate was added into the thimble before adding the potato sample. The results are reported in Table 3, and they were computed statistically. The mean chlorpropham values were found as 3.05, 3.07, 3.10, 3.25, and 2.93 mg/kg for the 5, 10, 20, 30, and 40 g samples with standard deviations of 0.73, 0.19, 0.12, 0.04, and 0.49, respectively (Table 3). The recoveries tended to increase with the increase in sample size to 30 mg but there was no significant difference among the values obtained from these different weights of sample even as low as 5 mg. However, the precision increased with increasing the amount recording up to 30 mg sample size. Although a sample with as low an amount as 5 g can be determined by this method with sufficient accuracy, yet a 30 mg sample is considered an optimum size for chlorpropham determination.

Table 4. Comparison of extraction techniques by estimating chlorpropham residues from treated samples provided by commercial store running at 8–10°C

Replication	Method of extraction	
	blending	reflux
1	2.36	2.85
2	2.62	2.87
3	2.12	2.79
4	2.56	2.85
5	2.77	2.87
Average recovery (mg/kg)	2.49	2.85
Standard deviation	0.25	0.03

After the requisite modification of the reflux method, the performance of both of the methods was again compared on potato samples taken from the conventional box stores. Five replicates of average weight of 30 mg for the reflux technique and 250 g for the blending method were run recovering 2.85 ± 0.03 mg/kg and 2.49 ± 0.25 mg/kg averaged values of chlorpropham, respectively (Table 4), the mean values being statistically significant ($P \leq 0.013$). It is now very much clear that the reflux technique performed equally well with the samples that were taken from the store. The technique has been proved to be a novel one giving highly reproducible result estimates. It has a capacity to manage easily a large number of storage samples in a wide range of sizes requiring chlorpropham analysis within the prescribed short period of time, and in comparison to the extraction process of Boyd *et al*. (1988), it offers overall better performance. The aspects of cost/expenditure have not been evaluated as this is outside the scope of the present study; however, we believe that the analysis made by our developed technique will not be more expensive than that one we are comparing it with.

CONCLUSIONS

In order to evaluate a large number of treated potato samples from commercial stores, a faster and more reliable technique is needed for chlorpropham determination. For this purpose, a method of reflux extraction technique was developed and compared with the blending extraction method adopted previously, and the efficiency of each technique was compared. After conducting several trials, the reflux extraction method was found to be quicker, more reliable, and advantageous over the

blending method. At least 20–30 samples can be analysed in a day by the use of reflux extraction device with greater accuracy as compared to 5–8 samples with the blending method. For the treated tuber samples, the reflux method was more reliable and precise with a standard deviation of 0.03. Whereas in the blending method, too many steps are involved raising its standard deviation to 0.25 (Table 4). The reflux extraction method does not require shaking the sample for at least 24 h in aluminium bottle neither the lengthy purification/clean up steps necessary for the blending technique. Further analytical improvement was made by optimising the sample quantity in the range of 5 g to 40 g giving results of high precision values. For being easier in handling and quicker in the determination, the reflux technique is a recommended analytical device for routine use.

Recommended technique

The final routine method developed during this trial is summarised below.

The washed or unwashed samples of treated potatoes from commercial stores were chopped with an electric food processor, and replicated 30 g sample from the homogenate was taken into a pre-weighed thimble with 10 g of anhydrous sodium sulphate added. The sample was then extracted with 150 ml of hexane for two hours in a reflux extracting unit in such mode as to allow the solvent vapours to pass round the thimble containing the sample for more rapid extraction. The collected extract from each replicate was then separately evaporated to dryness in a rotary evaporator under reduced pressure, re-dissolved in hexane with rinsing the flask several times with the solvent, and evaporated with dry nitrogen to make up the final volume to 2 ml. An aliquot (0.5 µl) was then injected into the GC operating with a glass column of 2 mm *i.d*, FID detectors at 230°C temperature, injector temperature of 220°C, oven temperature of 174°C, and nitrogen as a carrier gas at 25 cm^3/minute. The amount of chlorpropham was calculated comparing the GC values with those of the standard solution. The average of 5 replicates was calculated and considered as the mean chlorpropham residue level in the tubers. The recommended methodology of reflux extraction is simple and accurate, and works efficiently for the determination of chlorpropham, particularly when the analyses of a large number of samples are required from commercial potato stores necessary for a rapid feedback.

References

Ascenzo G., Gentili A., Marchese S., Mariono A., Perret D. (1998): Multiresidue method for determination of post-emergence herbicides in water by HPLC/ESI/MS in positive ionization mode. Environmental Science and Technology, **32**: 1340–1347.

Batora V., Vitorovic S.L.J., Their H.P., Klisenko M.A. (1981): Development and evaluation of simplified approaches to residue analysis. Pure and Applied Chemistry, **53**: 1039–1049.

Beernert H., Hucorne P. (1991): A simple and quick gas chromatographic method for the determination of propham and chlorpropham in potatoes. Zeitschrift für Lebensmittel-Untersuchung und -Forschung, **193**: 433–435.

Boyd I.M.G. (1988): Studies on the potato sprout suppressant chlorpropham. [Thesis.] University of Glasgow.

Chen Guoqiang, Pengying C., Liu Renjiang (2010): A multi-residue method for fast determination of pesticides in tea by ultra performance liquid chromatography-electrospray tandem mass spectrometry combined with modified QuEChERS sample preparation procedure. Food Chemistry, **125**: 1406–1411.

Corsini D., Stalknecht G., Sparks W. (1978): A simplified method for determining sprout-inhibiting levels of chlorpropham (CIPC) in potatoes. Journal of Agricultural and Food Chemistry, **26**: 990–991.

Heras A.P., Sanchez R. (1982): Determination of carbamic herbicides by high performance liquid chromatography (HPLC). II. Chlorpropham. Journal of Liquid Chromatography, **5**: 327–335.

Kadamne J., Proctor A. (2010): Rapid oil extraction from potato chips. Journal of American Oil Chemists' Society, **87**: 835–836.

Kerstholt R.P.V., Ree C.M., Moll H.C. (1997): Environmental life cycle analysis of potato sprout inhibitors. Industrial Crops and Products, **6**: 187–194.

Lewis D. J., Thorpe S.A., Reynolds S.L. (1996): The carry-through of residues of thiabendazole, tecnazene and chlorpropham from potatoes following manufacture into potato crisps and jacket potato crisps. Food Additives and Contaminants, **13**: 221–229.

Nakagawa H., Lockman J.C., Frankel W.L., Hampel H., Steenblock K., Burgart I.J., Thibodeau S.N., de la Chapelle A. (2004). Mismatch repair gene PMS2: disease-causing germline mutations are frequent in patients whose tumors stain negative for PMS2 protein,

but paralogous genes obscure mutation detection and interpretation. Cancer Research, **64**: 4721–4727.

Ritchie W., Boyed I.M.G., Duncan H.J. (1983): Method for the determination of chloro residues in crisps and crisps frying oil. Potato Research, **26**: 73–77.

Vliet V.W.F., Hertog S. (1966): The analysis of sprout inhibitor residues in the potato tuber. European Potato Journal, **9**:152–160.

Received for publication September 3, 2009
Accepted after corrections February 1, 2011

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