CLETHODIM (187)

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EXPLANATION

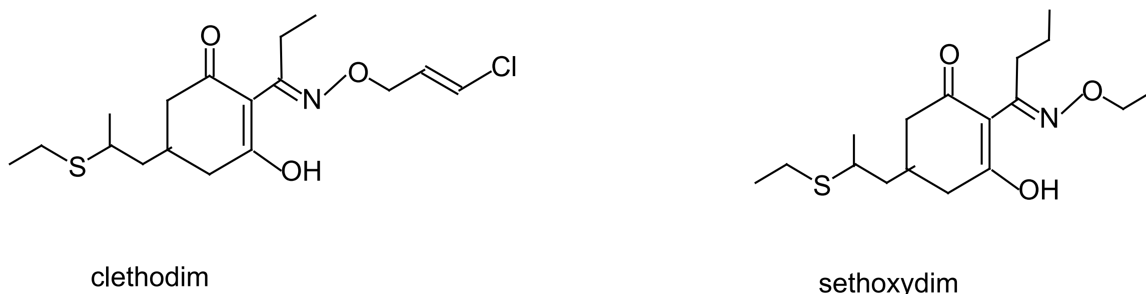
Clethodim was evaluated by the JMPR in 1994, 1997 and 1999. At the 2000 CCPR the governments of France, Germany and The Netherlands expressed concerns about the availability of an analytical method for regulatory purposes and the rather high and variable limits of determination in several commodities. At the 2001 CCPR the delegation from Germany questioned whether the notionally specific method of analysis used by the manufacturer could differentiate between residues arising from the use of clethodim and those from sethoxydim. The 2001 CCPR concluded that MRLs in the process of development should not be advanced in the absence of a suitable regulatory method. This was reaffirmed by the 2002 CCPR.

The 1999 JMPR recommended that the definition of the residue for compliance with MRLs and for the estimation of dietary intake should be the sum of clethodim and metabolites containing 5-(2-ethylthiopropyl)cyclohexene-3-one and 5-(2-ethylthiopropyl)-5-hydroxycyclohexene-3-one moieties and their sulfoxides and sulfones, expressed as clethodim.

Analytical aspects

Clethodim and sethoxydim share a common moiety, which accounts for the major part of their structures. Their structures differ in two parts: the oxime oxygen bears an ethyl group in sethoxydim but a 3-chloroallyl group in clethodim, and the imino carbon bears an *n*-propyl group in sethoxydim but an ethyl group in clethodim.

Figure 1. Structures of clethodim and sethoxydim.

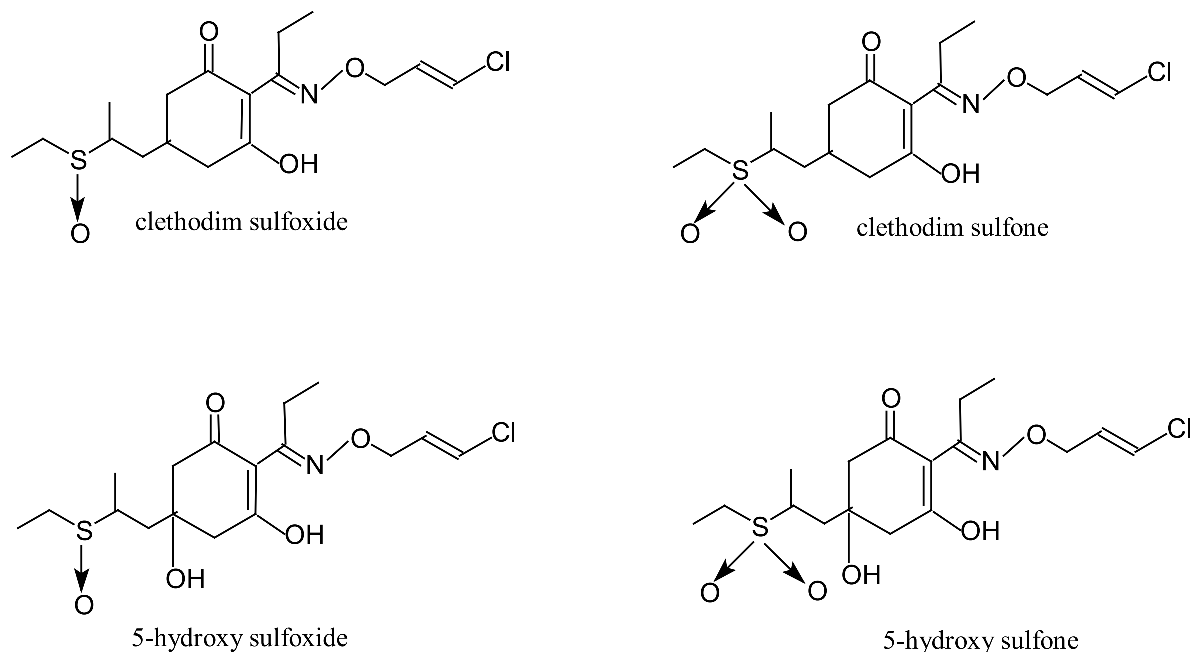


The proton on the 3-hydroxyl of the hydroxycyclohexenone moiety is mobile and keto-enol tautomerism occurs in both clethodim and sethoxydim. The two tautomers of each compound are indistinguishable.

In addition, *(E)* and *(Z)* isomers occur at the oxime nitrogen of clethodim. The *(E)* form is the less polar and they are readily separated by HPLC. However the two forms interconvert in aqueous solution fairly readily at pH 5 and 7 but not measurably at pH 9, and the interconversion may be observable in HPLC chromatograms by the slight tailing between the two peaks. Sethoxydim has no such separable isomers.

For the purposes of residues analysis, in addition to clethodim (a sulfide) itself, it is also necessary to determine the sulfoxide and sulfone, and also their 5-hydroxylated counterparts. Sethoxydim forms the corresponding sulfoxide and sulfone.

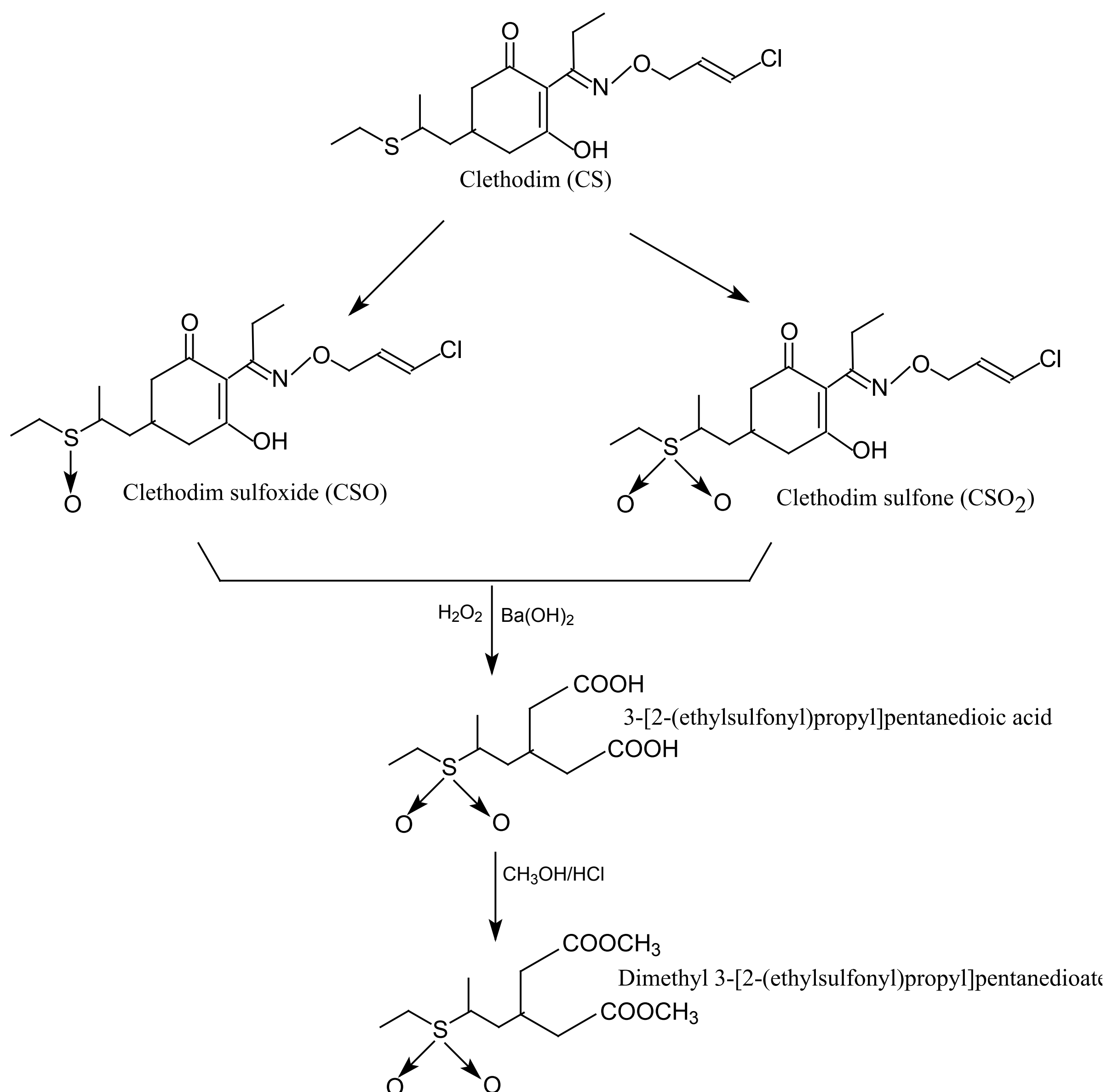
Figure 2. Structures of sulfoxides and sulfones of clethodim and 5-hydroxy-clethodim.



Both clethodim and sethoxydim have a chiral centre at the 2-carbon of the ethylthiopropyl group. Sulfoxides are also chiral and therefore the *(E)* and *(Z)* isomers of clethodim and sethoxydim sulfoxide should each exist as two diastereoisomers which in principle might be separable by achiral chromatography. The reverse-phase system (phenyl-hexyl column eluted with a methanol/water gradient containing a constant 0.1% of formic acid) used in the study by Reed (2002) appeared unlikely to separate the diastereisomers and the chromatograms showed no evidence of it.

Early studies on clethodim residues used an analytical method involving treatment with alkaline H_2O_2 to oxidise the sulfide and sulfoxide to the corresponding sulfone, simultaneously oxidatively cleaving the hydroxycyclohexenone ring to form 3-[2-(ethylsulfonyl)propyl]pentanedioic acid. The acid was methylated with anhydrous methanol and HCl for gas chromatography and detection by a flame-photometric detector in the sulfur mode.

Figure 3. Structures of compounds and reaction sequence in non-specific method.



3-[2-(ethylsulfonyl)propyl]pentanedioic acid is a photolysis product of clethodim which is not included in the definition of the residue but is also produced from sethoxydim under the same analytical conditions. The method is therefore not specific for the determination of clethodim.

The 1999 JMPR evaluation refers to a specific HPLC method (EPA-RM-26-D-3) having been developed for the determination of clethodim residues by Lai (1996). It was a modification of methods EPA-RM-26-D-1 (Lai and Ho, 1990) and EPA-RM-26-D-2 (Lai and Fujie, 1993). Detection is by UV absorption at 266 or 254 nm. The extracted residues are subjected to alkaline precipitation, the 5-hydroxyl is methylated with diazomethane and the sulfides and sulfoxides oxidised to sulfones with *m*-chloroperbenzoic acid. However the available description of the method is slightly ambiguous.

The "alkaline precipitation" and oxidation with *m*-chloroperbenzoic acid could be construed as being similar to the process used in the non-specific method, leading to the generation of 3-[2-(ethylsulfonyl)propyl]pentanedioic acid. Hence the method would be no more specific than the

original one. The lack of clarity regarding the "alkaline precipitation" presumably gave rise to the concerns about the specificity of the new method expressed at the CCPR.

The 1999 evaluation indicates that the methylated forms of clethodim, 5-hydroxy-clethodim sulfone, sethoxydim and 5-hydroxy-sethoxyodim sulfone produce four separated HPLC peaks, not two, suggesting that the common moiety is not produced. However UV absorption at a single wavelength (or even two) is not a specific detection technique.

A new method was submitted by the manufacturer for evaluation by the 2002 JMPR (Reed, 2002) based on LC-MS/MS (triple-sector quadrupole) detection with electropray ionisation in the positive ion mode. The alkaline precipitation involves the addition of solid calcium hydroxide to the aqueous methanol extract in the presence of Celite 545, brief swirling and immediate filtration, followed by acidification with HCl. The principle of this process (which is to be completed in <10 min at room temperature) is not described in the report but the manufacturer confirmed that it is a clean-up, intended to remove relatively strong organic acids from the extracts, while avoiding degradation of clethodim and its metabolites. Oxidation with *m*-chloroperbenzoic acid is intended only to oxidise the sulfides and sulfoxides to their corresponding sulfones. It does not generate 3-[2-(ethylsulfonyl)propyl]pentanedioic acid.

The resulting HPLC chromatograms clearly show the presence of two isomers (*E* and *Z*) of clethodim sulfone, 5-hydroxy-clethodim sulfone and S-methyl-clethodim sulfone (the last is not included in the definition of the residue). The isomer ratios were evidently not constant with some interconversion occurring on the column, but the two peak areas were summed in each case. The *(E)* and *(Z)* isomers of clethodim sulfone and 5-hydroxy-clethodim sulfone were detectable as four separated peaks.

The analytes were detected by multiple reaction monitoring (MRM) of the transitions *m/z* 392 to 164 for clethodim sulfone, *m/z* 408 to 204 for 5-hydroxy-clethodim sulfone, and *m/z* 378 to 164 for S-methyl-clethodim sulfone. The manufacturer confirmed that the precursor ions for each transition corresponded to the ^{35}Cl isotopic protonated molecule, $[M+H]^+$. Sethoxydim and its metabolites cannot produce these precursor ions and would also have different retention times and, presumably, produce much lower sensitivity under the same conditions. The product ions were not rationalised in the analytical method description but the manufacturer noted that the fragmentations produced by the clethodim sulfone and S-methylclethodim sulfone followed a pattern similar to that of clethodim, documented by Marek *et al*. (2000). The common fragment of m/z 164 (the most abundant fragment detected by these authors) is postulated to be generated from clethodim by [M - $OCH_2CH{=}CHCl$ - $CH_2CH(CH_3)SCH_2CH_3$]. By analogy the product m/z 164 is presumed to be generated from clethodim sulfone by [M - $OCH_2CH{=}CHCl$ - $CH_2CH(CH_3)SO_2CH_2CH_3$], and from S-methyl-clethodim sulfone by [M - $OCH_2CH{=}CHCl$ – $CH_2CH(CH_3)SO_2CH_3$]. Sethoxydim and sethoxydim sulfoxide were reported by Marek *et al.* to undergo a corresponding fragmentation to generate a product ion of m/z 178. Sethoxydim sulfone may therefore be expected to produce a product ion at m/z 178. The protonated molecule of sethoxydim sulfone cannot generate product ions of the same m/z ratio as those of clethodim. The fragmentation of 5-hydroxy-clethodim sulfone is evidently not analogous to that of clethodim.

LC-MS/MS of a single transition has the potential for interference from unrelated compounds, although the risk is not very great with relatively large molecules such as clethodim and its metabolites. Where the sulfones of clethodim and its metabolites are detected in a single sample by LC-MS/MS, the evidence of identity will be strongly supported if the four peaks are detected. However if only one of the sulfones is detected in a supposed residue of clethodim, additional specificity could be obtained by monitoring the corresponding transition of the ^{37}Cl isotopic protonated molecule involved. The acquisition of data for the transition(s) *m/z* 394 to 164, 410 to 204 and/or 380 to 164, would permit determination (albeit with only about one-third of the sensitivity) of the ion ratio for molecules containing a single chlorine atom and provide good supporting evidence of the identity of clethodim residues.

Even without this possible refinement it is clear that the method is highly selective towards, and under most circumstances will provide acceptable specificity for, clethodim.

The accuracy and precision achieved in recovery experiments with tomatoes, soya beans, soya bean oil, sugar beet roots and tops, beef kidney, liver, fat and muscle, chicken muscle and eggs, and cow's milk was determined in the range 0.01 to 0.5 mg/kg. Average recoveries (n = 5 for every combination) of clethodim, clethodim sulfoxide and 5-hydroxy-clethodim (measured as the sulfones) were in the range 50-117%, with RSDs in the range 3-20%. Low recoveries (34-43%) of 5-hydroxy clethodim occurred infrequently. A single and uncharacteristic zero recovery of clethodim could have been due to a mistake by the analyst. No false positives were detected in control samples. Given the relative complexity of the method and the nature of the determination procedure, the data appear generally satisfactory.

Limits of quantification (LOQs) ranged from 0.01 mg/kg for tomatoes to 0.1 for soya bean oil, with soya beans, tops and roots of sugar beet, beef muscle, fat, liver and kidney, cow's milk, and chicken muscle and eggs at 0.05 mg/kg. Recovery was determined at the LOQ and ten times that concentration. The nature of the detection technique and the LOQs recorded indicate that the method is likely to be sufficient for the determination of compliance with all proposed MRLs, including the values of 0.5 mg/kg(*) for beans, cotton seed oil, rape seed oil and soya bean oil, 0.2 mg/kg (*) for mammalian and poultry meat and offal, 0.1 mg/kg(*) for fodder beet and sunflower seed oil, and 0.05 mg/kg (*) for eggs and milk.

APPRAISAL

Clethodim {(±)-2-[(E)-1-[(E)-3-chloroallyloxyimino]propyl]-5-[2-(ethylthio)propyl]-3-hyroxycycl-ohex-2-enone} was evaluated by the JMPR in 1994, 1997 and 1999. At the 2001 CCPR, the delegation of Germany questioned whether the notionally specific method of analysis employed by the company could actually differentiate residues arising from the use of clethodim from those produced from sethoxydim. This followed concerns expressed by the delegations of France, Germany and The Netherlands at the 2000 CCPR about the availability of an analytical method for regulatory purposes and the rather high and variable limits of determination in several commodities. The 2001 CCPR concluded that the MRLs in development would not be advanced in the absence of a suitable regulatory method and this was reaffirmed by the 2002 CCPR.

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3-[2-(ethylsulfonyl)propyl]pentanedioic acid is a photolysis product of clethodim (which is not included in the residue definition) but it also produced from sethoxydim under the same analytical conditions. The method is therefore not specific for the determination of clethodim.

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including the values of 0.5 mg/kg(*) (for beans, cotton seed oil, rape seed oil, soya bean oil), 0.2 mg/kg (*) (for mammalian and poultry meat and offals), 0.1 mg/kg(*) (for fodder beet and sunflower seed oil) and 0.05 mg/kg (*) (for eggs and milk).

The 1999 JMPR evaluation referred to an HPLC method for the determination of residues of clethodim, which was described as being specific. Its ability to differentiate between residues of clethodim and sethoxydim was questioned at the 33rd and 34th sessions of the CCPR.

Consideration of the 1999 JMPR evaluation suggests that the method described would be capable distinguishing between the two pesticides but additional specificity (and perhaps sensitivity) is provided by a recent development by the manufacturer.

The new method also employs an HPLC separation but detection is by positive-ion electrospray LC-MS/MS. Validation of the new method with a range of fortified samples showed acceptable recoveries and limits of quantification.

Conclusions

The LC-MS/MS method is selective towards residues of clethodim and results for clethodim cannot be confused with sethoxydim.

For most purposes the specificity of the method should be sufficient to determine that the measurements relate to clethodim and not to an interfering compound but, if doubt remains, the transitions of the ^{37}Cl isotopic forms of the protonated molecules could also be monitored.

The LC-MS/MS method evidently has adequate sensitivity for control of compliance with proposed MRLs. It would be helpful if the company could identify the reasons for the occasional low recovery of 5-hydroxyclethodim sulfone.

The HPLC-UV absorption method evaluated by the 1999 JMPR appears to provide some specificity. The presence of the *(E)* and *(Z)* isomers of the sulfones in the LC-UV chromatogram would provide some support for the identification. Chromatographic separation from the corresponding metabolites of sethoxydim should avoid interference from that source.

REFERENCES

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