Appendix XII: Gas Chromatographic and High-Performance/Ultra-High Performance Liquid Chromatographic Fingerprinting

GC and HPLC/UHPLC fingerprinting refers to the identification of CMM samples by the examination of the GC and HPLC/UHPLC chromatograms of their solvent extracts.

GC and HPLC/UHPLC fingerprinting have the merits of high selectivity, high sensitivity, high separation rate and requiring only a short analytical time with a small amount of sample. In general, one or more chemical markers and characteristic peaks can be identified in the chromatographic fingerprinting.

Method – Establishment of a chromatographic fingerprinting

(1) Appropriate extraction method and chromatographic conditions should be selected in accordance with the nature of the active ingredients or markers contained in CMM samples. The chromatographic fingerprinting should contain as many well resolved peaks as possible in order to provide adequate information for the identification. The recommended run time for every GC and HPLC chromatographic fingerprinting is not more than 60 min.

(2) In the chromatographic fingerprinting established, a well resolved peak corresponding to an available chemical reference substance can be used as a marker peak for the calculation of the RRTs of other peaks in the same chromatogram. When necessary, more than one marker peak may be chosen. In general, the *R* value of the marker peak with adjacent peak must be larger than 1.0, unless otherwise specified.

(3) The RRT of a characteristic peak is calculated based on the retention time of a chosen marker peak by using the following equation –

$$\text{RRT} = \frac{\text{Retention time of the characteristic peak}}{\text{Retention time of the marker peak}}$$

(4) The reference fingerprint chromatogram, marker peak, characteristic peaks and acceptable ranges of a sample is drawn up based on the test results of at least 10 batches of representative samples examined in duplicate (i.e. a total of 20 sets of data).

(5) A fingerprint chromatogram of a sample is then established by using the above procedure. For positive identification, the sample must give all the characteristic peaks with the RRTs falling within the acceptable range of the corresponding peaks in the reference fingerprint chromatogram as specified in the individual monograph.