High performance liquid chromatography consists of the injection of a liquid sample into a liquid mobile phase which is passed through a column of solid or supported liquid stationary phase. Separation is the result of partition, adsorption, size exclusion or ion exchange between the stationary and mobile phases and the separated constituents of the sample are usually detected by an ultra-violet absorption detector. A flow chart of the typical instrumentation used is illustrated in figure D10.1.

*Flow chart of high performance liquid chromatography with chemiluminescence detection.*

Coupling with chemiluminescence detection adds the sensitivity of this technique to selectivity of a powerful separation method. It requires measurement of the emitted light due to a post-column reaction between the analytes in the column eluents and the chemiluminescence reagents, which are delivered by additional pumps with the incorporation of devices for rapid mixing. Measurement of the chemiluminescence intensity at its maximum requires optimization of the transit time (dependent on length of tubing and flow rate) between the mixing point and the detector. The most important problem in designing the coupling instrumentation is ensuring compatibility between the conditions necessary for efficient chromatographic separation and those needed for intense chemiluminescence. Separation depends heavily on mobile phase composition, whereas chemiluminescence emission is known to be affected by solvent, pH, reaction temperature and the presence of enhancers and/or catalysts

![Image of flow chart](image1.png)

*Figure D10.2 – Post-column instrumentation for measuring chemiluminescence after derivatization of analytes (PMT = photomultiplier tube; REC = recorder).*

Interfaces between chromatography columns and chemiluminescence can become very complex. For example, peroxy-oxalate chemiluminescence is frequently coupled with HPLC. As it detects only fluorescent analytes (see chapter B5), successful detection depends on derivatization of the analytes eluted from the column before the addition of the chemiluminescence reagents. Figure D10.2 shows part of the post-column arrangements used for the determination of catecholamines by peroxy-oxalate chemiluminescence after reaction with ethylene diamine, which produces fluorescent derivatives. For simplicity, the arrangements for different temperatures in different parts of the system have not been shown.